DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

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MECHANISM OF ACTION OF ANTIDEPRESSANTS: ASPECTS OF SEROTONINERGIC SYSTEM AND ITS INTERACTION WITH GLUTAMATE

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This dissertation is based on the following publications referred to by Roman numerals (I–VI) and some unpublished data:

I Skrebuhhova-Malmros T, **Pruus K**, Rudissaar R, Allikmets L, Matto V, Modulation of forced swimming, open field, and apomorphine-induced aggressive behaviour by 5-HT_{2A} and 5-HT_3 receptor ligands in male Wistar rats. Pharm Pharmacol Lett 1999; 2:70–73

II Skrebuhhova-Malmros T, Allikmets L, Rudissaar R, **Pruus K**, Matto V, Ondansetron fails to reverse antidepressant-elicited antiexploratory effects in the elevated plus-maze and open field tests. Med Sci Res 1999; 27:835–837

III Pruus K, Vaarmann A, Rudissaar R, Allikmets L, Matto V. Acute antidepressant treatment has no major effect on post-mortem brain monoamine content in rats subjected to forced swimming test. Pharm Pharmacol Lett 2001; 2:91–94

IV Pruus K, Rudissaar R, Vaarmann A, Matto V, Allikmets L. 1-(1-naphthyl)-piperazine, a mixed 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor ligand, elicits an anxiolytic-like effect in the open-field test without changes in 5-HT metabolism. Meth Find Exp Clin Pharmacol 2002; 24:151–157

V Pruus K, Vaarmann A, Rudissaar R, Allikmets L, Matto V. Role of 5-HT_{1A} receptors in the mediation of acute citalopram effects: a 8-OH-DPAT challenge study. Prog Neuropsychopharmacol Biol Psychiatry 2002; 26:227–232

VI Pruus K, Rudissaar R, Allikmets L, Harro J. Effect of acute combined treatment with antidepressants and the NMDA receptor antagonist MK-801 on forced swimming and open field activity in rats. (in manuscript)

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Paper I–II: Performed around half of the experiments. Participated in study design and writing of the manuscript.

Paper III–V: Performed all experimental work and half of the calculations. Helped to prepare the manuscript.

Paper VI: Performed half of the experimental work and calculations. Main person responsible for writing.

ABBREVIATIONS

1-NP	1-(1-Naphthyl)-piperazine HCl
5-HIAA	5-hydroxyindole-3-acetic acid
5-HT	5-hydroxytryptamine; serotonin
5-HTP	5-hydroxytryptophan
5-HTT	5-hydroxytryptamine transporter
8-OH-DPAT	(±)-8-hydroxy-dipropylaminotetralin HBr
AC	adenylyl cyclase
AMPA	5-methyl-4-isoxazole propionic acid
ANOVA	analysis of variance
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CREB	cAMP response element binding protein
DA	dopamine
DOI	(±)-2,5-dimethoxy-4-iodoamphetamine HCl
DSP-4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine HCl
ECS	electroconvulsive shock
GABA	γ-aminobutyric acid
HPLC-ECD	high performance liquid chromatography with electrochemical detection
i.p.	intraperitoneal(ly)
L-TRP	L-tryptophan
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
mCPBG	1-(m-chlorophenyl)-biguanide
mGluRs	metabotropic glutamate receptors
MK-801	(5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-10-imine maleate;dizocilpine
NA	noradrenaline
NARI	noradrenaline reuptake inhibitor
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
PCP	phencyclidine
p-CPA	para-chlorophenylalanine
PLC	phospholipase C
SSRI	selective serotonin reuptake inhibitor
TCA	tricyclic antidepressant
TWEEN 85®	polyoxyethylene-(20)-sorbitan oleate
WAY100635	N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide maleate

1. INTRODUCTION

Antidepressants are widely used in the treatment of depression, anxiety and panic disorder (Åsberg and Mårtenson, 1993; De Jonghe and Swinkels, 1997). Most of the antidepressants belong either to the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) or noradrenaline reuptake inhibitors (NARIs). The latter groups of antidepressants display considerably less side effects, thereby, especially the SSRIs, being drugs of choice in most cases (De Jonghe and Swinkels, 1997).

Serotonin (5-HT) and 5-HT receptors play an important role in many neuropsychiatric disorders. The tricyclic antidepressants with a serotonin-positive effect (such as nonselective noradrenaline and 5-HT reuptake inhibitors and selective 5-HT reuptake inhibitors) are used for the treatment of depression. Nevertheless, antidepressants have an important disadvantage: it is generally known that independently of their mechanism of action, all antidepressants must be administered for several weeks before they elicit a marked therapeutic effect. Recent evidence suggests that the drugs acting at 5-HT_{1A} and $5\text{-HT}_{2A/2C}$ receptors may be very useful for treatment of anxiety and psychotic disorders, respectively. Our main aim was to compare some aspects of mechanism of action of serotonergic and noradrenergic antidepressants: citalopram and fluoxetine, and desipramine and maprotiline, respectively.

2. REVIEW OF LITERATURE

2.1. Serotonin and its distribution in the central nervous system (CNS)

The existence of a vasoconstrictive substance in the blood has been known for over 135 years. The substance was named serotonin (5-hydroxytryptamine, 5-HT) and finally identified in 1949. The presence of 5-HT in the brain was reported by Gaddum in 1954. Gaddum also demonstrated that the action of 5-HT was antagonised by the potent hallucinogen lysergic acid diethylamide. Only 1–2% of the serotonin in the body is in the brain. But there is no equilibration between body serotonin and brain serotonin — the serotonin in the brain is independently synthesized from L-tryptophan transported across the blood-brain barrier. Serotonin can be synthesised both in the neural cell bodies and terminals. Serotonin is synthesised with conversion of the amino acid L-tryptophan to 5-hydroxytryptophan (5-HTP), which is then decarboxylated to serotonin. Tryptophan is hydroxylated by tryptophan hydroxylase to 5-HTP and it is the rate-limiting step in the synthetic pathway of serotonin. Serotonin is stored in vesicles. Following its release, serotonin is taken up into serotoninergic nerve terminals using of a specific Na^+/K^+ ATPase-dependent carrier — serotonin transporter (5-HTT). Once back in the nerve terminals, serotonin is either restored in vesicles or metabolised by MAO to 5-hydroxyindole-acetaldehyde followed by subsequent reduction to 5-hydroxyindole-acetic acid (5-HIAA) (Bradley, 1989).

The cell bodies of 5-HT neurones are located in the raphe nuclei in the brainstem and project to all areas of the brain, including the limbic system which is involved in the control of mood (Blier, 2003). It has been estimated that the human brain contains about 250 000 5-HT neurons of a total of 10^{11} neurons (Celada et al., 2004). In the CNS, serotonin has been implicated in regulation of sleep, mood, anxiety, cognition, aggression, appetite, temperature, sexual behaviour and pain processing. Depression is often associated with low synaptic levels of 5-HT.

5-HT is known to interact with other neurotransmitter systems. The activity of 5-HT neurons is controlled by a number of afferent pathways, mainly including glutamatergic inputs from forebrain areas such as the prefrontal cortex, a tonic noradrenergic input from various pontine nuclei and inhibitory γ-aminobutyric acid (GABA)-ergic inputs from local interneurons. Information about the role of other transmitters such as histamine or acetylcholine and peptides (e.g., substance P, corticotropin-releasing factor, cholecystokinin, hypocretin-orexin) is emerging (Celada et al., 2004). Very important mechanism of control of 5-HT neurons is self-inhibition through 5-HT_{1A} autoreceptors. Local release of 5-HT at cell bodies will thus diminish neuronal firing and produce a negative feedback regulation of transmitter release.

2.2. Serotonin receptor families

There are now seven families recognized (5-$HT_{1–7}$), comprising a total of 14 structurally and pharmacologically distinct mammalian 5-HT receptors subtypes (Hoyer et al., 1994; Barnes and Sharp, 1999). With the exception of the 5-HT_3 receptor, which is a ligand-gated ion chanel, 5-HT receptors belong to the G-protein-coupled receptor superfamily. The 5-HT_1 receptors are negatively coupled to adenylyl cyclase, the 5-HT_2 receptors are positively coupled to phospholipase C (PLC), and the 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 subtypes are positively coupled to adenylyl cyclase (AC) (Barnes and Sharp, 1999; Hoyer et al., 2002; Glennon, 2003).

2.2.1. 5-HT_1 receptor

The 5-HT_1 receptor class is comprised of five receptor subtypes that are negatively coupled to AC: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}.

5-HT_{1A} receptors have been found in high density in limbic brain areas, especially hippocampus and lateral septum, in cortex, and in dorsal and median raphe nuclei in the mesencephalon (Barnes and Sharp, 1999). 5-HT_{1A} receptors occur in mammalian brain in two different populations. 5-HT_{1A} receptors are located postsynaptically on the 5-HT nerve terminals, mainly in cortico-limbic areas, and presynaptically on the 5-HT neurons themselves at the level of the soma and dendrites in the midbrain raphe nuclei. On 5-HT neurons of the midbrain raphe nuclei receptors act as autoreceptors that control negatively 5-HT nerve firing and synthesis/release of 5-HT (Barnes and Sharp, 1999; Hoyer et al., 2002). The activation of 5-HT_{1A} receptors increases potassium conductance, thus hyperpolarizing the neuronal membrane and reducing the firing rate of serotonergic neurons in the cortex and hippocampus. Most antidepressant drugs increase the concentration of 5-HT in the extracellular brain space by preventing its reuptake. However, this increase is offset by a negative feedback operating at the 5-HT cell-body level.

The serotonin 5-HT_{1B} receptor is expressed in the CNS of rodents and its homologous 5-HT_{1D} receptor is expressed in human. 5-HT_{1B} receptors concentrated in the basal ganglia, striatum and frontal cortex and are thought to serve as terminal autoreceptors. In addition, the 5-HT_{1B} receptor may also act as terminal heteroreceptor controlling the release of other neurotransmitters, such as acetylcholine, glutamate, dopamine, noradrenaline, and γ-aminobutyric acid. These receptors are also found in cerebral arteries and other vascular structures (Barnes and Sharp, 1999; Hoyer et al., 2002; Sari, 2004).

2.2.2. 5-HT_2 receptor

5-HT_2 receptors are G-protein coupled receptors and coupled positively to PLC and mobilize intracellular calcium. 5-HT_2 receptors currently comprise three subtypes: 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors.

High level of 5-HT_{2A} receptors has been found in cortex, nucleus caudatus, nucleus accumbens, olfactory tubercle, and hippocampus (Lopez-Gimenez et al., 1997).

The 5-HT_{2B} receptors expressed in low levels in the brain (amygdala, lateral septum and hypothalamus), and at much higher levels in the placenta, lung, liver, kidney, heart, intestine, and stomach (Hoyer et al., 2002).

The 5-HT_{2C} binding sites are found in cortex, nucleus accumbens, hippocampus, amygdala, nucleus caudatus, and substantia nigra (Lopez-Gimenez et al., 2001).

The 5-HT_{2A} and 5-HT_{2C} receptors mediate effects of a large variety of compounds affecting depression, schizophrenia, anxiety, sleep patterns, feeding behaviour, sexual, and neuro-endocrine functions (Baxter et al., 1995).

2.2.3. 5-HT_3 receptor

The 5-HT_3 receptor is a ligand-gated cation channel. In the periphery, it is found on autonomic neurons and on neurons of the sensory and enteric nervous system. In the CNS, the 5-HT_3 receptors have been localized in the area postrema, nucleus tractus solitarii, nucleus caudatus, nucleus accumbens, amygdala, hippocampus, entorhinal, frontal, and cingulate cortex. 5-HT_3 receptors modulate the release of neurotransmitters and neuropeptides like dopamine, cholecystokinin, acetylcholine, GABA, substance P, and 5-HT itself. These receptors have been demonstrated to be involved in sensory transmission, regulation of autonomic functions, integration of the vomiting reflex, pain processing and control of anxiety (Farber et al., 2004).

2.2.4. Other classes of 5-HT receptors

The 5-HT_4 receptor is positively coupled to AC and exists in two isoforms (5-HT_{4S} and 5-HT_{4L}). The receptor is widely distributed in the CNS and peripheral tissues. 5-HT_4 receptors are able to modulate the release of monoamines and GABA in the brain. In the periphery, the receptor plays an important role in the function of several organs including the alimentary tract, urinary bladder, heart, and adrenal gland (Hegde and Eglen, 1996).

The 5-HT_5 receptor family consists of two members designated as 5-HT_{5A} and 5-HT_{5B}. To date the 5-HT_{5A} receptor has been identified in the mouse, rat, and human. The 5-HT_{5B} receptors are also expressed in the mouse and rat, but

not in the human. The 5-HT_5 receptors are located in the hypothalamus, hippocampus, corpus callosum, and glia (Hoyer et al., 2002), and the 5-HT_{5A} receptors have also been found on neurons and neuronal-like cells of the carotid body (Nelson, 2004).

The 5-HT_6 receptors are found in the striatum, amygdala, nucleus accumbens, hippocampus, cortex, and olfactory tubercle (Hoyer et al., 2002). The 5-HT_6 receptor appears to regulate glutamatergic and cholinergic neuronal activity, and increasing evidence suggests that it may be involved in the regulation of cognition, feeding and, possibly, affective states (Woolley et al., 2004). Many antipsychotics and antidepressants have high affinity for 5-HT_6 receptors and act as antagonists at this receptor (Hoyer et al., 2002).

The 5-HT_7 receptor has been identified in rat, mouse, human, pig, and guinea pig. Highest 5-HT_7 receptor densities are present in the thalamus and hypothalamus, and significant densities in the hippocampus and cortex. Although the biological functions of the 5-HT_7 receptors are poorly understood, preliminary evidence suggests that it may be involved in thermoregulation, learning and memory and sleep, and this receptor might be involved also in the regulation of mood. It has been suggested that the 5-HT_7 receptor is a putative target in the treatment of depression (Hedlund and Sutcliffe, 2004; Leopoldo, 2004; Thomas and Hagan, 2004). Acute restraint stress increases 5-HT_7 receptor mRNA expression in the rat hippocampus and chronic administration of the antidepressants induces a downregulation of this receptor (Sleight et al., 1995; Yau et al., 2001).

2.3. Mechanism of action of antidepressants

Alterations in noradrenergic, serotonergic and/or dopaminergic function in the CNS have been implicated in the pathophysiology of depression. A common action of many antidepressants is the inhibition of the reuptake of the biogenic amines into nerve terminals. The first-generation medications are effective because they enhance both serotonergic and noradrenergic mechanisms. The action of monoamine oxidase inhibitors (MAOIs) (e.g., moclobemide, phenelzine, tranylcypromine) is to increase the ability of the monoamine neurotransmitters NA, DA and 5-HT by inhibiting the enzyme MAO, blocking their metabolism and thus, increasing of neurotransmitter amount in the cytoplasm (Stahl, 1998). Tricylic antidepressants (TCAs) (e.g., desipramine, amitriptyline, imipramine, nortriptyline) work by inhibiting the reuptake of the neurotransmitters NA, DA or 5-HT by nerve cells. TCAs also block histaminic, cholinergic, and α_1-adrenergic receptor sites, and this action brings about unwanted side effects such as weight gain, dry mouth, constipation, drowsiness, and dizziness (Feighner, 1999; D'Aquila et al., 2000). Another important mechanism of the many classical antidepressants is the downregulation of

postsynaptic β_1-adrenergic receptors after chronic administration. Many antidepressants have been reported to produce changes in the regulation of 5-HT_1 and 5-HT_2 receptors administered chronically (Bodnoff et al., 1998).

The newest generation of antidepressants, including the selective serotonin reuptake inhibitors (SSRIs) (e.g., citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline), noradrenaline reuptake inhibitors (NARIs) (e.g., desipramine, maprotiline), and antidepressants with more the one site of action (e.g., venlafaxine, mirtazapine, nefazodone), target one or more specific brain receptor sites in most cases, without the unwanted effects such as on histamine, acetylcholine or noradrenaline receptors (Feighner, 1999; Kent, 2000). The mechanism of action of SSRIs is explained by selective inhibition of the serotonin transporter (Barker and Blakely, 1995). SSRI have a high ratio of 5-HT uptake inhibition compared to NA uptake inhibition. This is contrast to classical TCAs, which inhibit both 5-HT and NA uptake (Sambunaris et al., 1997). Furthemore, at variance with classical antidepressants, SSRIs may upregulate β_1-adrenergic receptors after chronic treatment (Palvimaki et al., 1994). After long-term administration of SSRIs, desensitization of postsynaptic serotonin receptors develops, and this may contribute to therapeutic action of SSRIs (Blier et al., 1987; Westenberg, 1999).

Recently, it has been discovered that drugs acting on some peptidergic systems have a considerable potential as antidepressants. Some neuropeptides (e.g., thyrotropin-releasing hormone, TRH) and antagonists of neuropeptide receptors (e.g., neurokinin NK_1 receptor) currently undergo clinical testing (Vetulani and Nalepa, 2000). The role of neuropeptides in the regulation of mood might result from the modulation of the action of monoamine transmitters with which they coexist in a neuron. Even more novel strategies, based on glutamatergic or GABAergic transmission or on intracellular messengers, are also explored. Converging lines of evidence indicate that the glutamatergic system might be a promising target for a novel antidepressant therapy. Both ionotropic glutamate receptor ligands (functional NMDA receptor antagonists and AMPA receptor potentiators) and compounds acting at metabotropic glutamate receptors (mGluRs; group I mGluR antagonists, group II antagonists and group III agonists) produce antidepressant-like activity in several preclinical and some clinical studies (Vetulani and Nalepa, 2000; Palucha and Pilc, 2005).

The slowness in development of the clinical effect of antidepressants suggests that acute alterations in monoamine metabolism alone cannot explain the entire antidepressant effect (Nestler et al., 2002) and indicate that antidepressants may act by evoking adaptive changes in intracellular signal transduction and synaptic connectivity (Duman et al., 1997; Altar, 1999; Skolnick, 1999; Manji et al., 2001; Reid and Stewart, 2001; Nestler et al., 2002). Various antidepressants may influence different intracellular signal cascades and alter their interrelationships, e.g., at the level of protein kinases. However, from the biological point of view, only the end-result of an antidepressant treatment counts, and it may be identical because of

convergence of the intracellular signals. This convergence occurs at the level of transcription factors, whose transcriptional activities are regulated by the convergent activities of various protein kinases such as the Ca^{2+}/calmodulin-dependent protein kinase II and others. Transcription factors recognize and bind to the specific sequences of DNA response elements, in the promotor regions of genes and change the rate of expression of a target gene. Various classes of antidepressants increase the expression of cAMP response element binding protein (CREB) (Schwaninger et al., 1995; Koch et al., 2003). Among the multiple target genes that could be regulated by CREB is brain-derived neurotrophic factor (BDNF) (Duman, 1998). BDNF has been shown to function as a key regulator of neurite outgrowth, synaptic plasticity and the selection of functional neuronal connections in the CNS (McAllister et al., 1999; Mamounas et al., 2000; Huang and Reichardt, 2001; Poo et al., 2001), which makes neurotrophins the potential mediators of the plastic changes induced by antidepressants (Duman et al., 1997; Altar, 1999; Manji et al., 2001; Nestler et al., 2002). For example, BDNF administration augments 5-HT metabolism (Siuciak et al., 1996, 1998) and stimulates serotonergic axonal growth in neocortex and spinal cord (Mamounas et al., 1995; Xu et al., 1995; Bregman et al., 1997). The enhancement of 5-HT neurotransmission by BDNF potentiates several behaviors regulated by 5-HT (Siuciak et al., 1994; Pelleymounter et al., 1995) and produces antidepressant effects in animal models of depression (Siuciak et al., 1997). Infusion of exogenous BDNF into hippocampus or brain stem has antidepressant-like behavioural effects (Shirayma et al., 2002), and BDNF administration increases serotonergic innervation (Mamounas et al., 2000) as well as the levels of 5-HT and its metabolites in forebrain (Siuciak et al., 1996). Chronic (but not acute) administration of several types of antidepressant drugs increases expression and activation of BDNF and its receptor TrkB (Nibuya et al., 1995; Russo-Neustadt et al., 2000) in rodent hippocampus and prefrontal cortex (Saarelainen et al., 2003; Castrén, 2004).

2.4. The serotonin hypothesis of major depression

5-HT is believed to play a multifunctional role in depression and a deficiency in brain serotonergic activity increases vulnerability to major depression. Abnormalities in serotonergic activity in depression could occur several levels, for example, diminished availability of L-tryptophan (L-TRP), the precursor of 5-HT, impaired 5-HT synthesis, release, reuptake or metabolism and or 5-HT postsynaptic receptor abnormalities. Available antidepressant drugs act by enhancing central serotonergic activity (Middlemiss et al., 2002). Which of the 5-HT receptor subtypes is primarily involved in the action of antidepressants is uncertain. Pineyro and Blier (1999) proposed that the dysregulation of the presynaptic and/or hetero- and autoreceptors of the 5-HT-ergic pathways might

be an important mechanism in the delayed clinical response of the antidepressants. In many works, where different neurochemical, electrophysiological, *in vivo*, and *post-mortem* methods were used, it has been found that the 5-HT_{1A} receptors are involved in the mechanism of action of antidepressants (Dawson et al. 1999; Gartside et al. 1999; Hajos et al. 1999; Haddjeri et al. 1999; Wolf et al. 1998; Currie et al. 1998; Salgado-Commissariat and Alkadhi, 1997). Somatodendritic 5-HT_{1A} receptors control firing of 5-HT neurons and stimulation of these receptors produces an inhibition of neuronal firing activity and subsequent reduction in 5-HT release. As the 5-HT autoreceptors have an inhibitory function on 5-HT release, it seems possible that the increase in serotonergic neurotransmission that follows chronic (but not acute) treatment with the antidepressants is particularly due to the desensitization of 5-HT autoreceptors (Goodwin, 1996; Stahl, 1998; Vaswani et al., 2003). Another way of overcoming the inhibitory feedback loop is to block the somatodendritic 5-HT_{1A} autoreceptors with an antagonist. Many studies have shown that acute challenge with a 5-HT_{1A} receptor antagonist potentiates the extracellular output of 5-HT after SSRI treatment (Dreshfield-Ahmad et al. 2000).

The 5-HT_{2A} receptors also appear to be involved both in the aetiology of depression and in the mode of action of antidepressants. Clinical studies have shown that depression could arise from a pathological increase in 5-HT_{2A} receptor function in limbic regions of the brain. The density of 5-HT_{2A} receptors is increased in the frontal cortex of the brain of suicide victims (Mann et al., 1986; Leonard et al., 2000). Most antidepressants decrease the number of receptors and decrease the activity of the phosphatidyl-inositol linked second messenger system following their chronic administration. Clinical studies have shown that the number of 5-HT_{2A} receptor sites on the platelet membrane of untreated depressed patients is significantly elevated (Pandey, 1997). However, the functional activity of these receptors is markedly reduced in untreated depressed patients but normalizes following effective antidepressant treatment.

It has been speculated that the 5-$HT_{1B/1D}$ receptors may have a role to play in depression and in the mechanism of action of antidepressants. These receptors appear to be located presynaptically where they control the release of 5-HT. The 5-$HT_{1B/1D}$ receptors are supersensitive in depression, thereby leading to a reduced intersynaptic concentration of 5-HT with a consequent increase in the number of postsynaptic 5-HT_2 receptor sites.

2.5. Relationships between serotonin and noradrenaline in the regulation of mood

It is well established that NA is involved in a whole range of physiological processes that are extremely important in the area of psychiatry: learning, memory, sleep, arousal, and adaptation (Leonard, 1997). The role of NA in depression and

stress reactions is linked to the neuroanatomical structure of the central noradrenergic system. Noradrenergic neurons project widely throughout the brain, innervating many regions involved in regulation of mood and adaptation. Many of cell bodies of NA neurons are located in the locus coeruleus and project to all parts of the brain. NA and 5-HT neurons interact at the level of their cell bodies in the bodies in the raphe nuclei and locus coeruleus, and they both project to the same neurons in the forebrain (Blier and de Montigny, 1997; Harro and Oreland, 2001; Brunello et al., 2002; Blier, 2003). Antidepressant treatments have been shown to enhance 5-HT and NA neurotransmission in the pyramidal cells in the rat hippocampus (Malberg et al., 2000; Malberg and Schechter, 2005). The NA and 5-HT neuronal cell bodies possess autoreceptors (the α_2-adrenoreceptor and 5-HT_{1A} receptor) which, when stimulated, inhibit the firing of their respective neurons. The firing of 5-HT neurons is also under the tonic excitatory control of a monosynaptic NA projection to the raphe nucleus and is mediated by α_1-adrenoreceptors on the raphe cell bodies. This synapse is also regulated by inhibitory α_2-adrenergic autoreceptors on the NA terminals which, when stimulated, reduce the release of NA and hence the firing of 5-HT cells in the raphe. The raphe and the locus coeruleus projections to the hippocampus are mainly inhibitory (via post-synaptic 5-HT_{1A} receptors and α_1-adrenoreceptors located on pyramidal neurons). The 5-HT neurons of the dorsal raphe also project to the locus coeruleus where they inhibit neuronal activity via an excitatory postsynaptic 5-HT_{2A} receptor located on a GABA neuron (Szabo and Blier, 2001; Blier, 2003). This 5-HT nerve terminal also carries an excitatory presynaptic kainate receptor sensitive to the neurotransmitter glutamate, which tonically promotes 5-HT release (Van Bockstaele, 2000).

2.6. Glutamatergic mechanisms in depression and in the action of antidepressants

Glutamate is the main excitatory neurotransmitter in the CNS with particularly prominent pathways innervating the cortex, hippocampus, striatum, septum, and amygdala. Many of these areas play a role in cognitive and emotional function. Some studies have suggested that glutamatergic system may also be involved in the etiology of depression (Petrie et al., 2000; Krystal et al., 2002; Paul and Skolnick, 2003). Postmortem studies have documented changes in the N-methyl-D-aspartate (NMDA) receptor complex in the frontal cortex and hippocampus of suicide victims (Nowak et al., 1995). Both preclinical and clinical studies indicate that compounds which reduce transmission at NMDA receptors have antidepressant-like properties. Animal studies have suggested that many antidepressant drugs show activity at the NMDA receptor and that NMDA antagonists have antidepressant-like profiles in preclinical models (Trullas and Skolnick, 1990; Skolnick, 1999; Petrie et al., 2000). There is also

evidence that glutamate also directly regulates synaptic activity of other neurotransmitters (Petrie et al., 2000). In the raphe, NMDA induces inhibitory postsynaptic currents (Jolas and Aghajanian, 1997) suggestive that antagonism of this receptor would increase raphe neural activity (Lejeune et al., 1994) and increase 5-HT release (Martin et al., 1998). An increase in phencyclidine-induced (PCP) extracellular 5-HT output is seen with microdialysis in the medial prefrontal cortex and dorsal hippocampus (Martin et al., 1998). Furthemore, other reports indicate that noncompetitive NMDA receptor antagonists, MK-801 and PCP, alter either 5-HT or 5-HIAA content in prefrontal cortex, the hippocampus and other brain sites (Loscher et al., 1993; Lillrank et al., 1994). Chronic treatment with NMDA antagonists resulted in antidepressant-like behavioural effects in such models as chronic mild stress, learned helplessness, footshock-induced aggression, and olfactory bulbectomy (Paul and Skolnick, 2003). Chronic, but not acute treatment with NMDA receptor antagonists, similar to classical antidepressants, induced the downregulation of forebrain β-adrenoreceptors (Paul et al., 1992). MK-801, a noncompetitive NMDA receptor antagonist, elicits locomotor hyperactivity in rats (Andine et al. 1999), and antidepressants increased the MK-801-induced locomotor hyperactivity (Maj et al., 1991; 1996). Antidepressants do not increase locomotor activity on their own, thus it is of interest whether there is any effect of combined administration of antidepressants with such a level of reduction of NMDA receptor function which does not lead to locomotor activation.

The adaptive changes of NMDA receptor induced by chronic antidepressant administration are reflected at the level of gene expression. Electroconvulsive shock (ECS) increases the mRNA coding for some NMDA receptor subunits (NR_{2A} in many regions) but decreased that for mGluR5b. Generally, long-term antidepressant treatment produces region-specific changes in expression of transcripts for NMDA receptor subunits, presumably altering NMDA receptor composition (Watkins et al., 1998).

The NMDA receptor is a ligand-dependent Ca^{2+} channel, and therefore regulates Ca^{2+} influx and, consequently, nitric oxide (NO) synthesis and thus, some effects may results from changes in intracellular Ca^{2+} and NO concentration. There are also data that Ca^{2+} antagonists, similarly to antidepressants, may downregulate the strychnine-insensitive glycine receptors (Nowak et al., 1993). It has also been found that NO synthase (NOS) inhibitors, similarly to NMDA receptor antagonists, produce antidepressant-like effect in the forced swimming test in mice and may have a potential as antidepressant agents (Harkin et al., 1999). On the other hand, chronic ECS increases the NOS activity in the cerebral cortex, cerebellum and hippocampus, and this may be regarded as a compensatory mechanism to counteract the reduced NMDA receptor complex reactivity (Nowak et al., 1997).

3. AIMS OF THE STUDY

The general objective of the present work was to study the role of the serotoninergic and glutamatergic mechanisms in the action of two groups of antidepressants — SSRIs and NARIs. The specific aims were the following:

1. Evaluation and comparison of effects of serotonin- and noradrenergic antidepressants (citalopram, fluoxetine, desipramine, maprotiline) and their concomitant administration with 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3 receptor ligands in tests of exploratory behaviour.

2. Evaluation and comparison of effects of serotonin- and noradrenergic antidepressants antidepressants (citalopram, fluoxetine, desipramine, maprotiline) and their concomitant administration with 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3 receptor ligands in forced swimming test (FST) in rats.

3. Evaluation and comparison of effects of antidepressants and their concomitant administration with 5-HT_{1A}, 5-HT_{2A}, and 5-HT_3 ligands on monoamine levels in the rat brain.

4. Elucidation of the role of NMDA receptors in the effects of antidepressant in animal experiments.

4. MATERIALS AND METHODS

4.1. Animals and laboratory conditions

Male and female Wistar rats (from Kuopio National Animal Centre, Kuopio, Finland) weighing 200–350 g were used in all experiments. The animals were housed under standard laboratory conditions four per cage with the exception of the *in vivo* microdialysis experiments, where the animals were housed singly. Water and food were always available *ad libitum*. The animal room had a controlled temperature (20°C±2°C) and a light/dark cycle (light on from 8.00 a.m. to 8.00 p.m.).

The number of animals per drug treatment group in the behavioural experiments was 8, in the postmortem experiments was 5 or 8, and in the *in vivo* microdialysis experiments 3 (for the vehicle-treated group) or 4 (for the drug-treated group). One hour before an experiment the animals were moved in their home cages from animal room into the behavioural testing room, unless stated otherwise. The experimental protocols were approved by The Ethics Committee of the University of Tartu.

4.2. Drugs and their administration

In the behavioural experiments, the following drugs were used:

1) antidepressants: citalopram (from Lundbeck A/S, Copenhagen, Denmark), fluoxetine and desipramine (both from Sigma, St. Louis, MO, USA), maprotiline and trazodone (both from Tocris, London, UK),
2) 5-HT_{1A} receptor agonists: 8-OH-DPAT (from Tocris, London, UK or from Sigma, St. Louis, MO, USA), 1-NP (from Sigma, St. Louis, MO, USA),
3) 5-HT_{1A} receptor antagonist: WAY 100635 (from Tocris, London, UK),
4) 5-HT_{2A} receptor agonist: DOI (from RBI Chemicals, Natick, MA, USA),
5) 5-HT_{2A} receptor antagonists: ketanserin, ritanserin (both from RBI Chemicals, Natick, MA, USA),
6) 5-HT_3 receptor agonist: mCPBG (from RBI Chemicals, Natick, MA, USA),
7) 5-HT_3 receptor antagonist: ondansetron (from Glaxo Wellcome, Indianapolis, IN, USA),
8) NMDA receptor antagonist: MK-801 (from Tocris, London, UK),
9) others: DSP-4 (from RBI Chemicals, Natick, MA), p-CPA (from Sigma, St. Louis, MO, USA).

The standards of 5-HT and 5-HIAA, and monobasic sodium phosphate were obtained from Sigma (St. Louis, MO, USA). Perchloric acid and sodium disulfite were purchased from Ridel-deHaën AG (Seelze, Germany), octanesulfonic acid sodium salt was from Fluka Chemie (Buchs, Switzerland) and HPLC grade methanol from Rathburn Chemicals Ltd. (Walkerburn, Scotland).

Citalopram, fluoxetine, desipramine, maprotiline, trazodone, 8-OH-DPAT, 1-NP, WAY 100635, DOI, and mCPG were dissolved in distilled water, ketanserin was dissolved in distilled water by addition of minimal amount of HCl. Ritanserin was suspended with few drops of Tween 80 and diluted with distilled water. Ondansetron was diluted to give dose of 4 mg/kg. All drugs in most of experiments were administered 30 min prior to an experiment i.p. In case of concomitant treatment with two drugs, these were injected intraperitoneally into opposite regions of abdomen.

DSP-4 was dissolved *ex tempore* in distilled water in a dose of 50 mg/kg and injected i.p. The DSP-4 pretreated animals were included into the behavioural experiments not earlier than after one week. p-CPA was suspended with a few drops of Tween 80 and diluted with distilled water and was administered in a single dose of 350 mg/kg i.p., 48 h before the behavioural experiment.

4.3. Behavioural methods

4.3.1. Open field test

In most of experiments a wooden, grey painted arena 100 x 100 cm with 40 cm sidewalls was used. On the test day, one hour before the experiment the animals were moved into the testing room. After drug treatment (30 min before test) the animals were returned to the home cage. For the test, the animal was placed into one of the central squares and observed during for 4 min for (1) horizontal (number of line crossings on the floor) and (2) vertical activity (number of rears). On the basis of these criteria (3) the sum of exploratory events was calculated. The horizontal activity was counted only if the animal crossed the line with four paws. Vertical activity was counted when the animal removed the forepaws from ground and stretched itself. In the experiments with acute MK-801 a metal quadrate arena 50 x 100 cm with 40 cm sidewalls was used. The surface of the floor was divided into eight squares of equal size.

4.3.2. Elevated plus-maze test

The elevated plus-maze apparatus consists of a maze with two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 30 cm) with an open roof. The arms were placed so that the two open and two enclosed arms were opposite to each other. All parts of the apparatus were made of wood and the apparatus was elevated 50 cm above the floor. The surface of an open arm was divided by dark lines into three parts of equal size. For a test (240 seconds), the animal was placed facing an enclosed arm and was observed for the following criteria: (1) the time of latency (the time of the first entry with all four paws from an

enclosed arm into an open arm); (2) the number of entries into the open arms; (3) the total number of entries; (4) the number of line crossings in the open arms; and (5) the time spent exploring in the open arms. The criteria (1) to (4) were scored only if the animal had moved with all four paws across a line. The criterion "time spent exploring in the open arm" was measured when the animal explored with two paws across a line.

4.3.3. Social interaction test

The larger open-field was used for this test as the arena. The observation time was 10 minutes. Two animals from different cages and with no prior contact were injected always with the same drug and were placed singly into empty cages. After 30 min separation, the animals were placed pair-wise onto the central squares of the apparatus. The time of social interaction was measured only when the animals were in active social contact (sniffing, crawling, climbing, following, active tactile contacting etc.), and passive interaction not recorded. The total interaction time of each animal pair was recorded (*i.e.*, the number of measures taken was four per treatment group).

4.3.4. Forced swimming test

The technique was first described by Porsolt and colleagues (1978; 1979). The experimentally naive rats were forced to swim for 15 min on the first day (the immobility time during the first five minutes period was recorded) and 5 min on the second day in a vertical glass cylinder (∅ 20 cm; height 60 cm) containing 40 cm of water maintained at 25°C. The duration of immobility was measured only when the rat was judged to be immobile, *i.e.* whenever it remained passively floating in the water in a slightly hunched but upright position, its head just above the surface. The injections of the vehicle and drugs were given immediately after the first swimming session and 30 min before the second swimming session.

In the experiments with acute MK-801 we used only one experimental session. Rats were forced to swim for 5 min in the vertical glass cylinder and the duration of immobility was measured. The injections of the vehicle and drugs were given 30 min before the swimming session.

The water was changed after observation of each rat.

4.4. Measurement of monoamine neurotransmitters and their metabolites in tissue samples

Immediately after the completion of the forced swimming test or social interaction test, the animals were moved to the biochemical laboratory and killed by decapitation. The skulls were opened and the brains were quickly removed and prepared on an ice-cold plate. Four structures were prepared: frontal cortex, striatum, hippocampus and hypothalamus. The brain samples were stored at –80°C until assayed.

HPLC-ECD (high performance liquid chromatography with electrochemical detection) analysis was performed with a Coulochem Electrode Array System (CEAS, Model 5600; ESA, Inc., Bedford, MA, USA) equipped with two Model 582 pumps and Model 540 autoinjector. Two coulometric array cell modules, each containing four electrochemical detector cells, were used. The analytical column (150 × 3.0 mm i.d.) used was a stainless-steel column packed with 3 µm particles of silica-based C_{18} materials (MD-150/RP- C_{18}, ESA). The column and detectors were housed in a thermal chamber maintained at 30°C. The system was controlled and the data were acquired and processed using the CoulArray software on a Pentium-based computer.

The mobile phase was made of 10% (v/v) methanol in 0.1 M monobasic sodium phosphate, 0.55 mM octanesulfonic acid with pH 3.10. The buffer solution was filtered through 0.2 µm GHP Polypro filters (Gelman Laboratory, Ann Arbor, MI, USA) and degassed under vacuum for 10 min. The flow-rate was 0.5 ml/min and the cell potentials (*versus* palladium reference) constituted an increasing array: 0 mV at electrode 1, 50 mV at electrode 2, with increments of 100 mV at each subsequent electrode until a value of 650 mV.

The frozen brain samples were weighed and then sonicated for 30 s in 300–1000 µl of ice-cold 0.12 M perchloric acid ($HClO_4$) containing 0.1% sodium disulfite ($Na_2S_2O_5$) and 5 ng/ml 3,4-dihydroxy-benzylamine (DHBA) as an internal standard. After centrifugation (20 min at 4°C, 13,400 *g*) 30 µl of supernatant was injected into the HPLC system. The primary stock standard solutions were made by dissolving 10–20 mg of the component in 25 ml 0.12 M perchloric acid. These concentrates were stored in 1 ml portions at -20°C and thawed when necessary at 4°C. Secondary standard solutions were made by dilution to give a concentration of 2–4 µM. Working standards in nM range were made freshly every day. Correct identification of the peak was obtained from the retention time (± 4%) and the relative ratio (at least 0.75) of the peak height measured with two or three electrodes at different voltages. Quantification of the compound was based on the peak area obtained for an external standard.

4.5. In vivo microdialysis procedure

Under chloral hydrate anaesthesia (350 mg/kg, i.p., diluted in distilled water that served as vehicle) the guide cannula for the microdialysis probe (Agn Tho's AB, Lidingö, Sweden) was implanted into the prefrontal cortex according to the coordinates taken from the Paxinos and Watson brain atlas of rat (1986): AP +3.4 mm, DV –6.0 mm, L –2.5 mm relative to bregma, and secured using 3 screws and dental base material. The standard surgical technique was used. After surgery, the animals were accommodated in to individual cages for 5–7 days.

In the morning of the experiment, rats were moved into the testing room (room temperature about 25°C) and anaesthetised using chloral hydrate (350 mg/kg, i.p.). During the whole procedure, the animals were kept under light anaesthesia: the depth of anaesthesia was adjusted with additional injections of chloral hydrate in such a way that the tendonal reflexes were present but no movements of the animal were allowed. The average dose of chloral hydrate used during the six-hours procedure was 154.4±6.1 mg per animal.

The microdialysis probe (the exposed tip was 4 mm, from Agn Tho's AB, Lidingö, Sweden) was inserted into the guide cannula and connected via the polyethylene tubing to a 1 ml microsyringe and modified Ringer solution (NaCl 147.0, KCl 2.7, $CaCl_2$ 1.2, $MgCl_2$ 1.0, ascorbic acid 0.02 mM) was infused through the microdialysis probe with a microinjector pump (2 μl/min). After two hours stabilization period the samples were collected every 15 min. The first four samples were considered as a baseline, which average value was taken as 100 per cent.

4.5.1. Measurement of 5-HT and 5-HIAA content in microdialysis samples

The same equipment and method as described above for the *post-mortem* experiments was used with following changes. A model 5014B microdialysis cell and a first CoulArray detector cell were set in series. The mobile phase consisted of 50 mM monobasic sodium phosphate, 0.50 mM sodium acetate, 0.42 mM octanesulphonic acid and 10% (v/v) of methanol, pH was adjusted to 4.10. The flow-rate was 0.5 ml/min and the cell potentials (*versus* palladium reference) constituted an increasing array: –100 mV at electrode 1,375 mV at electrode 2,400 mV at electrode 3 and 500 mV at electrode 4.

4.5.2. Verification of the location of the microdialysis probe

After completion of the experiment, the animals were killed under chloral hydrate anaesthesia by neck dislocation and the brains were removed from the skulls. The frozen brains were dissected using blades and the localization of the microdialysis probe was verified *in situ* without staining. Animals with instable baseline or wrong probe location were excluded from analysis.

4.6. Data analysis and statistics

Data from the behavioural and *post-mortem* experiments were subjected to analysis of variance (ANOVA) (factor: drug treatment). Whenever a significant drug treatment effect was found, the data were further analysed using the Fisher's LSD test, Student`s t-test, Kolmogorov-Smirnov two sample test or Newman-Keuls Multiple Comparison Test.

Data from the *in vivo* microdialysis experiments were subjected to repeated measures analysis of variance (repeated measures ANOVA), (factors: drug treatment and time between 15 and 180 min). Whenever a significant drug treatment effect or drug treatment × time interaction was found, the data were further analysed by separate time points using the ANOVA followed by the Fisher's LSD test (factor: drug treatment).

The probability levels $p<0.05$ were always considered statistically significant. All data are expressed as means±S.E.M.

5. RESULTS

5.1. Effect of antidepressants and compounds acting via 5-HT_{1A}, 5-HT_{2A}, or 5-HT_3 receptors on rat behaviour (Papers I–V, previously unpublished results)

5.1.1. Open field test

All antidepressants were injected 30 min before the experiment. The data on the exploratory events are summarised in Table 1. In the open field test, citalopram, desipramine, fluoxetine, and maprotiline (all 0–15 mg/kg) treatment elicited dose dependent attenuation of the number of line crossings, rearings, and the sum of exploratory events. In comparable dosages the citalopram effect was weakest. Desipramine, maprotiline and fluoxetine inhibited exploratory activity in two-threefold. It is known, that fluoxetine has also quite a significant adrenopositive effect. Thus adrenopositive antidepressants have a well pronounced inhibitory action after acute administration on exploratory behaviour. This could also be considered as an anxiogenic-like action.

The 5-HT_{1A} receptor agonist 8-OH-DPAT (0.1 mg/kg), while not affecting the horizontal activity, decreased the number of rears in the open field test, and had an additive effect on rearings with desipramine [$F(3,20)=3.8$, $p<0.05$] (Figure 1), and citalopram (Table 2) The effects of 8-OH-DPAT and citalopram were similar in the p-CPA pretreated groups (Table 2).

Table 1. The effect of acute antidepressant treatment in the open field test.

Drug treatment	Line crossings	Rearings	Sum
Vehicle	58 ± 3	14 ± 3	72 ± 3
Citalopram 5 mg/kg	65 ± 4	13 ± 2	77 ± 5
Citalopram 10 mg/kg	48 ± 6*	9 ± 2*	56 ± 6*
Citalopram 15 mg/kg	51 ± 4	6 ± 1**	57 ± 5*
Vehicle	45 ± 6	15 ± 2	60 ± 8
Fluoxetine 5 mg/kg	47 ± 6	11 ± 1*	59 ± 6
Fluoxetine 10 mg/kg	20 ± 3**	4 ± 1***	24 ± 3***
Fluoxetine 15 mg/kg	16 ± 5**	2 ± 1***	18 ± 6***
Vehicle	67 ± 6	20 ± 4	87 ± 9
Desipramine 5 mg/kg	37 ± 3***	9 ± 2**	48 ± 5***
Desipramine 10 mg/kg	32 ± 4***	12 ± 3*	44 ± 6***
Desipramine 15 mg/kg	19 ± 4***	6 ± 2***	25 ± 5***
Vehicle	64 ± 4	19 ± 2	82 ± 6
Maprotiline 5 mg/kg	50 ± 6	18 ± 3	68 ± 6
Maprotiline 10 mg/kg	36 ± 5**	7 ± 2**	43 ± 6**
Maprotiline 15 mg/kg	26 ± 4***	5 ± 1***	31 ± 5***

All data are expressed as means±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (Fisher's LSD test).

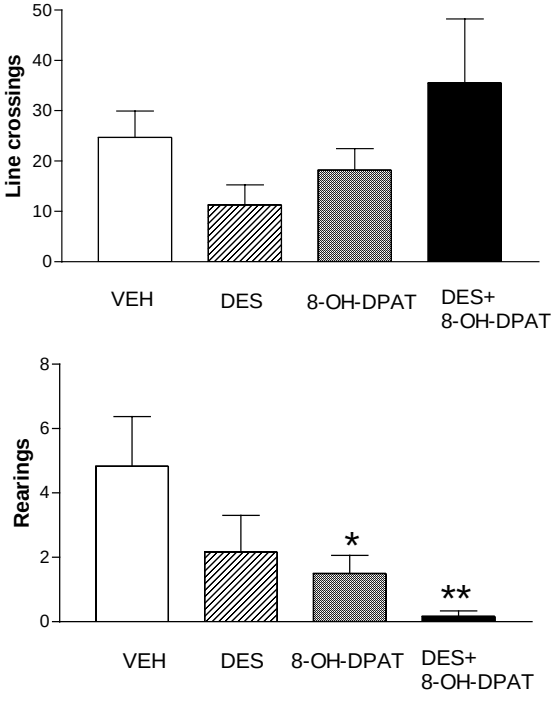

Figure 1. Effects of 5-HT_{1A} agonist 8-OH-DPAT and desipramine in the open field test. VEH = vehicle, DES = desipramine 10 mg/kg, 8-OH-DPAT 0.1 mg/kg, DES + 8-OH-DPAT = desipramine 10 mg/kg + 8-OH-DPAT 0.1 mg/kg. All data are expressed as means±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ (Fisher's LSD test).

Table 2. Effect of acute citalopram and 8-OH-DPAT treatment on the open field behaviour in rats

Pretreatment	Drug treatment	Line crossings	Rearings
vehicle	vehicle	45.5 ± 9.7	12.5 ± 3.3
vehicle	citalopram 5 mg/kg	40.8 ± 6.9	5.4 ± 1.4**
vehicle	8-OH-DPAT 0.1 mg/kg	44.1 ± 6.4	2.6 ± 0.8***
vehicle	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	54.5 ± 9.5	5.8 ± 0.9***
p-CPA	vehicle	34.7 ± 5.3	9.8 ± 1.8
p-CPA	citalopram 5 mg/kg	22.9 ± 2.2	4.7 ± 1.1**
p-CPA	8-OH-DPAT 0.1 mg/kg	24.5 ± 4.3	4.5 ± 1.1***
p-CPA	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	40.4 ± 3.9	2.7 ± 0.9***

All data (N per drug treatment group = 8) are expressed as means±SEM. $^{**}P<0.01$, $^{***}P<0.001$ as compared with the respective pretreatment + vehicle group (*i.e.*, vehicle + vehicle or p-CPA + vehicle group), Fisher's LSD test.

The 5-HT_{1A} receptor agonist and 5-$HT_{2A/2C}$ receptor antagonist 1-NP (2 mg/kg) elicited a significant effect on the number of line crossings [$F(3,28)=28.6$, $p<0.001$] and on the number of rearings [$F(3,28)=19.9$, $p<0.001$] (increase and decrease, respectively) in the open field test, which were not modified by citalopram (5 mg/kg) or desipramine (10 mg/kg) administration (Table 3 and Figure 2). Thus 1-NP effect could be explaned as an anxiolytic action, elicited through diminishing 5-HT output and blockade of 5-HT_2 receptors. Antidepressants are not able to change this effect because release of 5-HT is diminished.

Table 3. Effect of the 5-HT_{1A} agonist and 5-$HT_{2A/2C}$ receptor antagonist 1-NP and its co-administration with citalopram in open field test.

Drug treatment	Line crossings	Rearings	Sum
Vehicle	43.9 ± 4.2	13.1 ± 1.4	57.0 ± 4.6
1-NP 2 mg/kg	92.6 ± 6.6***	8.3 ± 1.5*	100.9 ± 7.0***
1-NP 2 mg/kg+ citalopram 5 mg/kg	104.6 ± 9.8***	3.0 ± 1.0***	107.6 ± 9.1***

All data are expressed as means±S.E.M. $^{*}p<0.05$, $^{***}p<0.001$ (Fisher's LSD test).

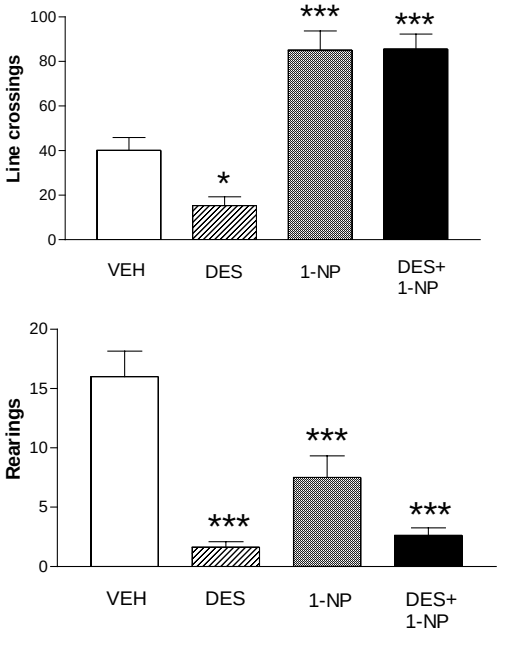

Figure 2. Effect of the 5-HT_{1A} agonist and 5-$HT_{2A/2C}$ receptor antagonist 1-NP and its co-administration with desipramine in open field test. VEH = vehicle, DES = desipramine 10 mg/kg, 1-NP 2 mg/kg, DES + 1-NP = desipramine 10 mg/kg + 1-NP 2 mg/kg. All data are expressed as means±S.E.M. $^{*}p<0.05$, $^{***}p<0.001$ (Fisher's LSD test).

The 5-HT_{1A} receptor antagonist WAY 100635 (0.3 mg/kg) increased the number of line crossings (Figure 3 and 4), and the number of rearings (Figure 4) in open field test measured in two different experiments. Desipramine (10 mg/kg) treatment decreased exploratory behaviour, citalopram (10 mg/kg) and fluoxetine (10 mg/kg) decreased the number of rearings and this effect was not reversed by WAY100635. Thus the effect of adrenergic antidepressant desipramine differs from the action of SSRIs. The significant antagonism of desipramine (also fluoxetine) could be explained through adrenopositive action.

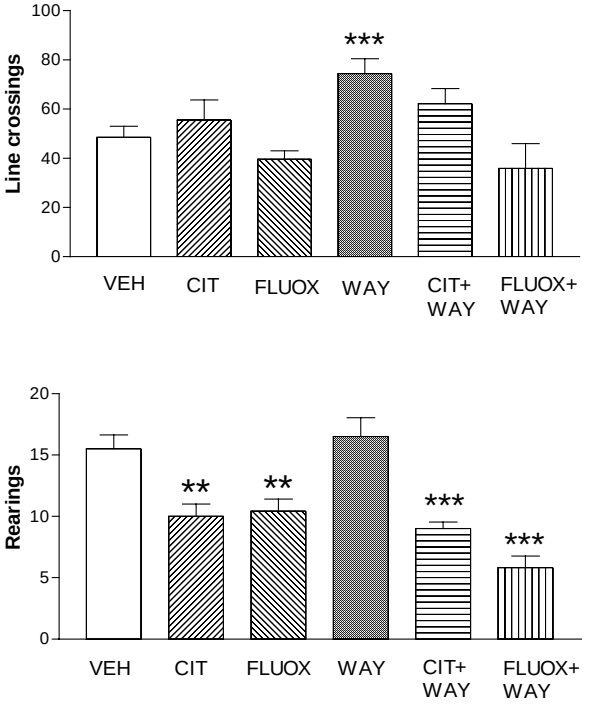

Figure 3. Effects of 5-HT_{1A} antagonist WAY 100635 and antidepressants in the open field test [$F(5,50)=5.3$, $p<0.01$, $F(5,41)=10.1$, $p<0.001$ for line crossings and rearings, respectively]. VEH = vehicle, CIT = citalopram 10 mg/kg, FLUOX = fluoxetine 10 mg/kg, WAY = WAY 100635 0.3 mg/kg, CIT + WAY = citalopram 10 mg/kg + WAY 0.3 mg/kg, FLUOX + WAY = fluoxetine 10 mg/kg + WAY 0.3 mg/kg. All data are expressed as means±S.E.M. $^{**}p<0.01$, $^{***}p<0.001$ (Fisher's LSD test).

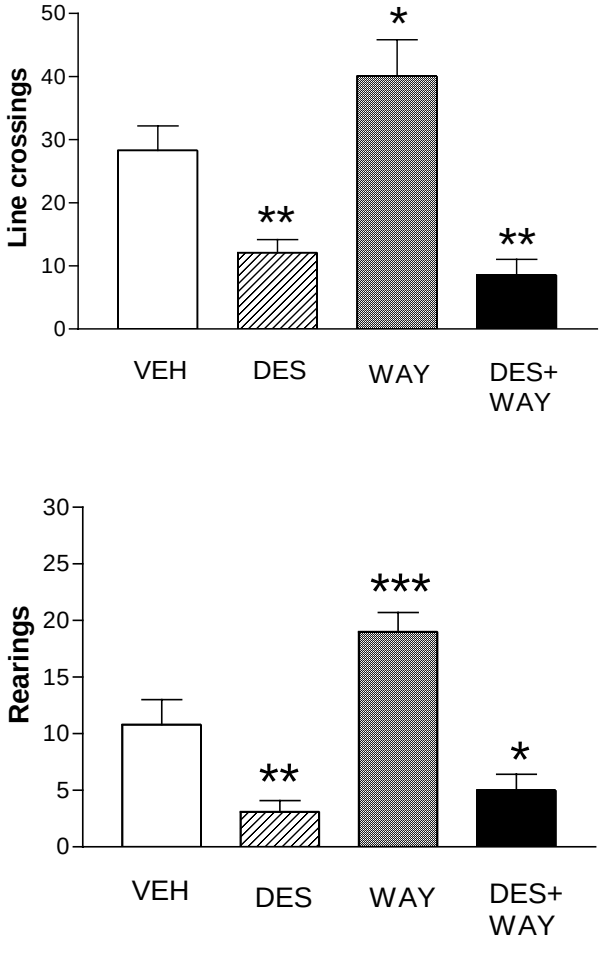

Figure 4. Effect of the 5-HT_{1A} antagonist WAY 100635 and the effect of desipramine in the open field test [$F(3,31)=16.98$, $p<0.001$, $F(3,30)=13.5$, $p<0.001$ for line crossings and rearings, respectively]. VEH = vehicle, DES = desipramine 10 mg/kg, WAY = WAY 100635 0.3 mg/kg, DES + WAY = desipramine 10 mg/kg + WAY 0.3 mg/kg. All data are expressed as means±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (Fisher's LSD test).

The 5-HT_{2A} receptor agonist DOI (1.5 mg/kg) which had no effect of its own (data not shown) decreased in combination with citalopram (5 mg/kg) the number of rears in the open field test [$F(2,17)=3.8$, $p<0.05$] (Figure 5). The serotonergic antidepressants are not able to antagonize the effect of DOI, because SSRIs increase the extracellular levels of 5-HT which has similar to DOI action on 5-HT_2 receptors.

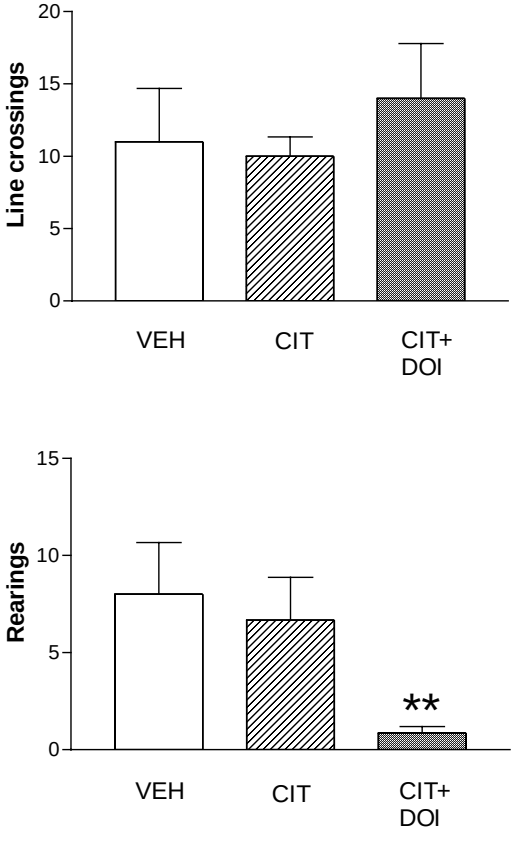

Figure 5. Effect of 5-HT_{2A} agonist DOI on the effect of citalopram in open field test. VEH = vehicle, CIT = citalopram 10 mg/kg, CIT + DOI = citalopram 10 mg/kg + DOI 1.5 mg/kg. All data are expressed as means±S.E.M. $^{**}p<0.01$ (Fisher's LSD test).

The 5-HT_3 receptor antagonist ondansetron (4 mg/kg) did not influence open field behaviour. Citalopram (5 mg/kg) decreased the number of rears and ondansetron did not change this effect of citalopram. Desipramine alone had a strong tendency toward the reduction of the number of line crossings, although this was statistically insignificant. In combination with ondansetron desipramine decreased the number of line crossings and rearings (Table 4).

Table 4. Effect of acute antidepressant and ondansetron treatment on the open field behaviour in rats

Drug treatment	Line crossings	Rearings
Vehicle	16.8 ± 4.6	9.51 ± 1.83
Citalopram 5 mg/kg	14.0 ± 4.6	4.16 ± 0.70*
Ondansetron 4 mg/kg	11.5 ± 2.8	7.83 ± 1.24
Citalopram 5 mg/kg + ondansetron 4 mg/kg	11.3 ± 1.8	4.00 ± 0.96*
Vehicle	14.8 ± 3.9	8.34 ± 0.88
Desipramine 10 mg/kg	7.5 ± 2.9	6.33 ± 3.73
Ondansetron 4 mg/kg	18.1 ± 4.1	9.66 ± 2.44
Desipramine 10 mg/kg + ondansetron 4mg/kg	7.1 ± 1.5**	1.66 ± 0.42**

All data (N per drug treatment group = 6) are expressed as means±SEM. *P<0.05 as compared with the respective vehicle-treated group; **P<0.05 as compared with the respective ondansetron-treated group (Dunn's test).

5.1.2. Plus-maze test

In the elevated plus-maze test, ANOVA revealed a significant effect on the following criteria: 1) the number of the closed arm entries, [$F(8,51)=3.05$, $p<0.01$]; 2) the number of line crossings on the open arms, [$F(8,51)=3.4$, $p<0.01$]; 3) the number of open arm entries, [$F(8,51)=2.79$, $p<0.05$]; and 4) the number of total arm entries, [$F(8,51)=4.5$, $p<0.001$]. The open/total arm entries ratio, time spent on open arms as well as open/total time ratio were unchanged. The 5-HT_{2A} antagonists ketanserin and ritanserin did not change exploratory behaviour in the plus-maze test. Desipramine alone and in combination with ketanserin and ritanserin decreased the number of closed arm entries, the number of total arm entries, and the number of line crossings on the open arms. Citalopram treatment was ineffective, but in combination with 5-HT_{2A} antagonists elicited antiexploratory effect (Table 5). Thus, adrenergic antidepressant desipramine has after acute administration well pronounced anxiogenic-like action, which is not antagonised by 5-HT_2 antagonists. This differs from the effect of SSRIs.

5-HT_3 antagonist ondansetron significantly increased the time spent on open arms and the percentage of time spent on open arms, which seems to be an anxiolytic-like action. Acute antidepressant treatment decreased the exploratory behaviour and this effect was not reversed by the ondansetron challenge (Table 6).

Table 5. Effects of 5-HT_{2A} antagonists ketanserin and ritanserin, and antidepressants in plus-maze test.

Drug treatment	Entries into closed arms	Entries into open arms	Total entries	Open/total entries ratio	Line crossings	Time spent on open arms	Open/total time ratio
Vehicle	4.58 ± 0.73	2.75 ± 0.27	7.33 ± 0.82	2.92 ± 0.33	20.41 ± 2.32	75.5 ± 14.6	31.4 ± 6.0
Desipramine 10 mg/kg	1.66 ± 0.55**	2.16 ± 0.30	3.83 ± 0.70**	1.75 ± 0.28	9.16 ± 1.32**	90.5 ± 24.0	37.7 ± 10.0
Citalopram 5 mg/kg	3.50 ± 0.84	3.50 ± 0.76	7.00 ± 1.39	2.06 ± 0.41	18.16 ± 5.30	68.8 ± 15.1	28.6 ± 6.3
Ketanserin 3 mg/kg	3.33 ± 0.80	2.66 ± 0.55	6.00 ± 0.89	2.82 ± 0.87	19.66 ± 4.99	66.5 ± 16.0	27.7 ± 6.6
Ritanserin 3 mg/kg	3.50 ± 0.67	2.83 ± 0.60	6.33 ± 0.91	2.65 ± 0.53	12.00 ± 1.98	63.5 ± 16.6	26.4 ± 6.9
Desipramine 10 mg/kg + ketanserin 3 mg/kg	1.66 ± 0.97**	2.00 ± 0.44	3.66 ± 0.95**	2.37 ± 1.13	6.50 ± 1.97***	82.6 ± 21.7	34.4 ± 13.2
Desipramine 10 mg/kg + ritanserin 3 mg/kg	0.66 ± 0.40***	1.33 ± 0.61*	2.00 ± 0.93***	1.61 ± 0.45	5.50 ± 2.55***	75.3 ± 21.8	31.3 ± 17.4
Citalopram 5 mg/kg + ketanserin 3 mg/kg	2.16 ± 0.54*	1.33 ± 0.21*	3.50 ± 0.56**	2.83 ± 0.55	10.50 ± 4.48*	42.5 ± 13.2	17.7 ± 5.5
Citalopram 5 mg/kg + ketanserin 3 mg/kg	2.00 ± 0.57*	1.33 ± 0.21*	3.33 ± 0.66**	2.58 ± 0.45	11.00 ± 1.52*	35.6 ± 8.1	14.8 ± 3.3

All data are expressed as means±S.E.M. *$p<0.05$, **$p<0.01$, ***$p<0.001$ (Fisher's LSD test).

Table 6. Effects of 5-HT_3 antagonist ondansetron and antidepressants in plus-maze test.

Drug treatment	Entries into open arms	Total entries	Line crossings	Time spent on open arms	Percentage time spent on open arms
Vehicle	1.67 ± 0.76	3.66 ± 1.02	10.17 ± 3.31	23.0 ± 10.2	9.58 ± 4.26
Citalopram 5 mg/kg	1.18 ± 0.54	2.83 ± 0.91	8.66 ± 2.34	15.5 ± 6.4**	6.45 ± 2.67**
Ondansetron 4 mg/kg	1.50 ± 0.56	2.67 ± 0.71	10.33 ± 3.07	51.3 ± 11.3*	21.38 ± 4.70*
Citalopram 5 mg/kg + ondansetron 4 mg/kg	0.66 ± 0.49	2.50 ± 0.84	5.66 ± 2.09	10.0 ± 4.4**	4.16 ± 1.83**
Vehicle	1.63 ± 0.54	3.66 ± 1.38	8.66 ± 4.18	21.1 ± 9.8	8.82 ± 3.71
Desipramine 10 mg/kg	0.83 ± 0.16	2.81 ± 0.60	7.16 ± 1.60	7.8 ± 3.2**	3.24 ± 1.34**
Ondansetron 4 mg/kg	2.00 ± 0.51	4.50 ± 0.72	15.00 ± 3.21	48.8 ± 9.4*	20.34 ± 3.94*
Desipramine 10 mg/kg + ondansetron 4 mg/kg	0.50 ± 0.22	1.50 ± 0.42**	4.66 ± 1.60**	13.0 ± 5.8**	5.41 ± 2.45*

All data are expressed as means±S.E.M. *$p<0.05$ as compared with the respective vehicle-treated group; **$p<0.05$ as compared with the respective ondansetron-treated group (Dunn's test).

5.1.3. Social interaction test

In the social interaction test, there were no statistically significant differences between the vehicle-treated, 1-NP-treated and 1-NP plus citalopram-treated groups. A moderate tendency toward the prolongation of social interaction as a consequence of 1-NP treatment was found (Table 7).

Table 7. Effect of the 5-HT_{1A} agonist and 5-$HT_{2A/2C}$ receptor antagonist 1-NP and its co-administration with citalopram in the social interaction test (Fisher's LSD test).

Drug treatment	Time in social interaction (s)
Vehicle 1 ml/kg	260 ± 27
1-NP 2 mg/kg	307 ± 93
1-NP 2 mg/kg+ citalopram 5 mg/kg	292 ± 85

5.1.4. Forced swimming test

On the second day of the experiment, desipramine (10 mg/kg) but not citalopram (5–10 mg/kg) treatment reduced the time of immobility in the FST.

The 5-HT_{1A} receptor agonist 8-OH-DPAT (0.1 mg/kg) significantly reduced the time of immobility in citalopram-treated rats both after vehicle and p-CPA pre-treatment. 8-OH-DPAT, while reducing immobility itself [$F(3,20)=6.5$, $p<0.01$], co-administered with desipramine led to no change in immobility (Table 8 and Figure 6).

Table 8. Effect of acute citalopram and 8-OH-DPAT treatment on the forced swimming behaviour in rats

Pretreatment	Drug treatment	Time immobile
vehicle	vehicle	182 ± 9.7
vehicle	citalopram 5 mg/kg	204 ± 12.1
vehicle	8-OH-DPAT 0.1 mg/kg	171 ± 8.9$^{**\ \$}$
vehicle	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	197 ± 8.0
p-CPA	vehicle	179 ± 10.3
p-CPA	citalopram 5 mg/kg	202 ± 8.7
p-CPA	8-OH-DPAT 0.1 mg/kg	183 ± 15.4$^{**\ \$}$
p-CPA	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	199 ± 10.1

All data (N per drug treatment group = 8) are expressed as means±SEM. $^{**}P<0.01$, as compared with the respective pretreatment + citalopram 5 mg/kg group (*i.e.*, vehicle + citalopram 5 mg/kg or p-CPA + citalopram 5 mg/kg group), $^{\$}P<0.05$ as compared with the respective citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg (*i.e.*, vehicle + citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg or p-CPA + citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg), Fisher's LSD test.

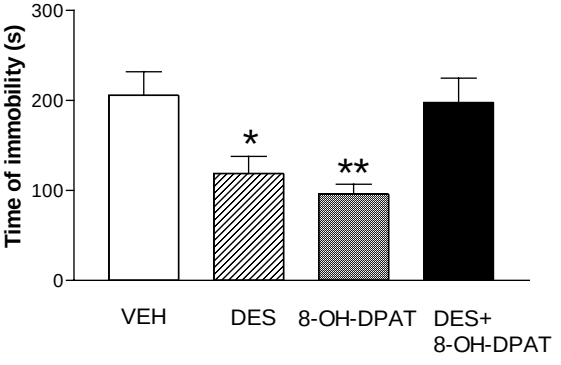

Figure 6. Effect of 5-HT_{1A} agonist 8-OH-DPAT on the effect of desipramine on the forced swimming behaviour in rats. VEH = vehicle, DES = desipramine 10 mg/kg, 8-OH-DPAT 0.1 mg/kg, DES + 8-OH-DPAT = desipramine 10 mg/kg + 8-OH-DPAT 0.1 mg/kg. All data are expressed as means±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ (Fisher's LSD test).

The 5-HT_{1A} receptor antagonist WAY 100635 (0.3 mg/kg) was ineffective in altering the time of immobility, although in combination with fluoxetine (10 mg/kg) and desipramine (10 mg/kg) the reducing effect on the time of immobility was found [$F(7,65)=7.2$, $p<0.001$] (Figure 7). We can conclude, that NARI desipramine, and SSRI fluoxetine which has also considerable adreno-positive effect, act similarly in FST. The pure SSRI citalopram does not change the effect of WAY because it also increases the level of 5-HT in synaptic cleft.

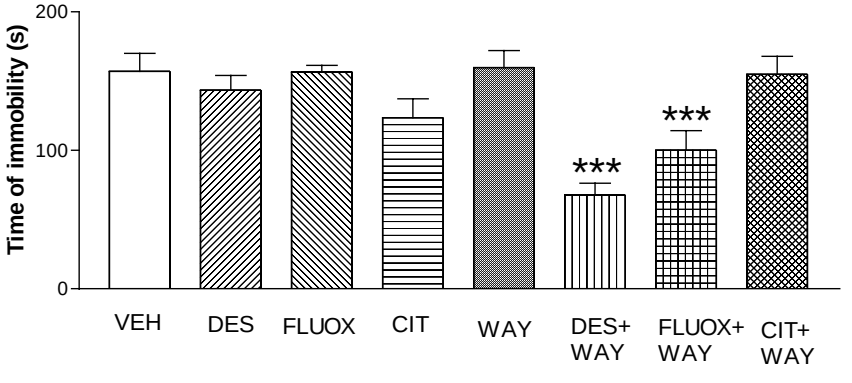

Figure 7. Effect of co-administration of 5-HT_{1A} antagonist WAY 100635 with antidepressants in the forced swimming test. VEH = vehicle, DES = desipramine 10 mg/kg, FLUOX = fluoxetine 10 mg/kg, CIT = citalopram 10 mg/kg, WAY = WAY 100635 0.3 mg/kg, DES + WAY = desipramine 10 mg/kg + WAY 100635 0.3 mg/kg, FLUOX + WAY = fluoxetine 10 mg/kg + WAY 0.3 mg/kg, CIT + WAY = citalopram 10 mg/kg + WAY 0.3 mg/kg. All data are expressed as means±S.E.M. $^{***}p<0.001$ (Fisher's LSD test).

The 5-HT_{2A} receptor agonist DOI (3 mg/kg) elicited an antidepressant-like effect in the forced swimming test. Citalopram (5 mg/kg) treatment was ineffective but it is noteworthy that citalopram was partially able to reverse the effect of DOI (Table 9).

Table 9. The effect of DOI, mCPBG, ondansetron, and citalopram on rat forced swimming.

Drug treatment	Time immobile
Experiment I: F(3,27)=18.8, p<0.0001	
vehicle	212.6 ± 10.1
citalopram 5.0 mg/kg	200.8 ± 7.9
DOI 3.0 mg/kg	64.1 ± 13.4 ***
citalopram 5.0 mg/kg + DOI 3.0 mg/kg	122.1 ± 25.6 **$
Experiment II: F(3,28)=0.90, NS	
vehicle	199.8 ± 15.9
citalopram 5.0 mg/kg	210.3 ± 8.9
mCPBG 10 mg/kg	199.8 ± 8.3
citalopram 5.0 mg/kg + mCPBG 10 mg/kg	196.9 ± 17.6
Experiment III: F(3,28)=1.29, NS	
vehicle	144.3 ± 4.8
citalopram 5.0 mg/kg	134.8 ± 11.7
ondansetron 4.0 mg/kg	152.0 ± 11.3
citalopram 5.0 mg/kg + ondansetron 4.0 mg/kg	125.4 ± 11.1

All values (in seconds) are data (±S.E.M.) obtained from the forced swimming experiments, subjected to the one-way ANOVA followed by Newman-Keuls Multiple Comparison Test. **p<0.01; ***p<0.001 drug treatment group vs. corresponding vehicle group. $p<0.05 citalopram 5.0 mg/kg + DOI 3.0 mg/kg vs. DOI 3.0 mg/kg alone. NS = not significant.

5-HT_{2A} receptor antagonists ketanserin (3 mg/kg) and ritanserin (3 mg/kg) did not influence the reducing effect of desipramine (10 mg/kg) on immobility, and did not change immobility either in combination with citalopram (Figure 8). In the DSP-4 pre-treated animals, neither desipramine nor citalopram in combination with 5-HT_{2A} receptor antagonists reduced the time of immobility (data not shown). This indicates that the reduction of immobility time in FST is depending on adrenomimetic action — in DSP-4 treated animals adrenergic terminals are destroyed.

The 5-HT_3 receptor agonist mCPBG (10 mg/kg) and 5-HT_3 receptor antagonist ondansetron (4 mg/kg) were ineffective in the FST (Table 9).

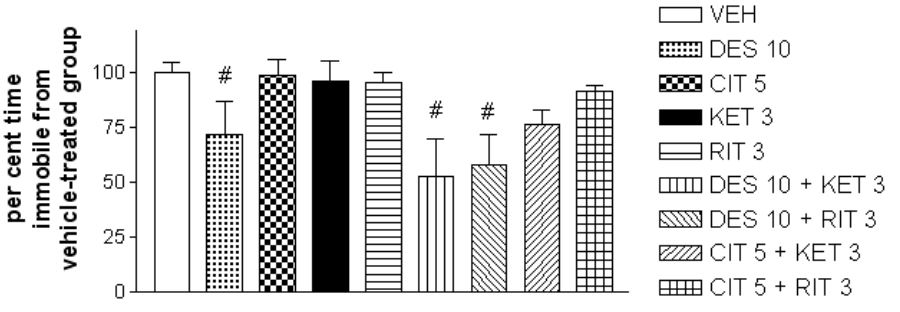

Figure 8. The effect of antidepressants, 5-HT_{2A} receptor antagonists, and their combined administration on the time of immobility in the forced swimming test. VEH — vehicle; DES 10 — desipramine 10 mg/kg; CIT 5 — citalopram 5 mg/kg; KET 3 — ketanserin 3 mg/kg; RIT 3 — ritanserin 3 mg/kg treatment. All data are expressed as means±S.E.M. $^{\#}p<0.05$ (Kolmogorov-Smirnov test)

5.2. Effects of antidepressants, NMDA antagonist MK-801 and their combined administration on rat behaviour (Paper VI)

5.2.1. Open field test

Acute administration of MK-801 at doses 0.025, 0.05 and 0.1 mg/kg did not show any significant effect in the open field test (Figure 9).

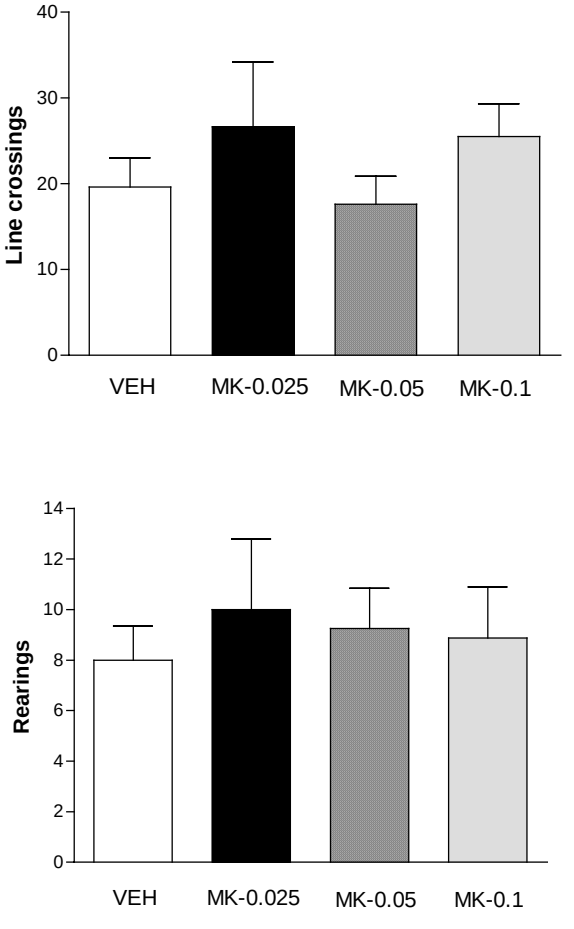

Figure 9. Effect of MK-801 (0.025, 0.05, 0.1 mg/kg) treatment on rat exploratory behaviour in the open field test. All data are expressed as means±S.E.M. (Fisher's LSD test).

According to post-hoc tests after a significant ANOVA [$F(3,24)=5.1$, $p<0.01$], combination of fluoxetine and MK-801 increased locomotor activity. Other antidepressants in combination with MK-801 had no significant effect on activity at the doses examined (Figure 10–11).

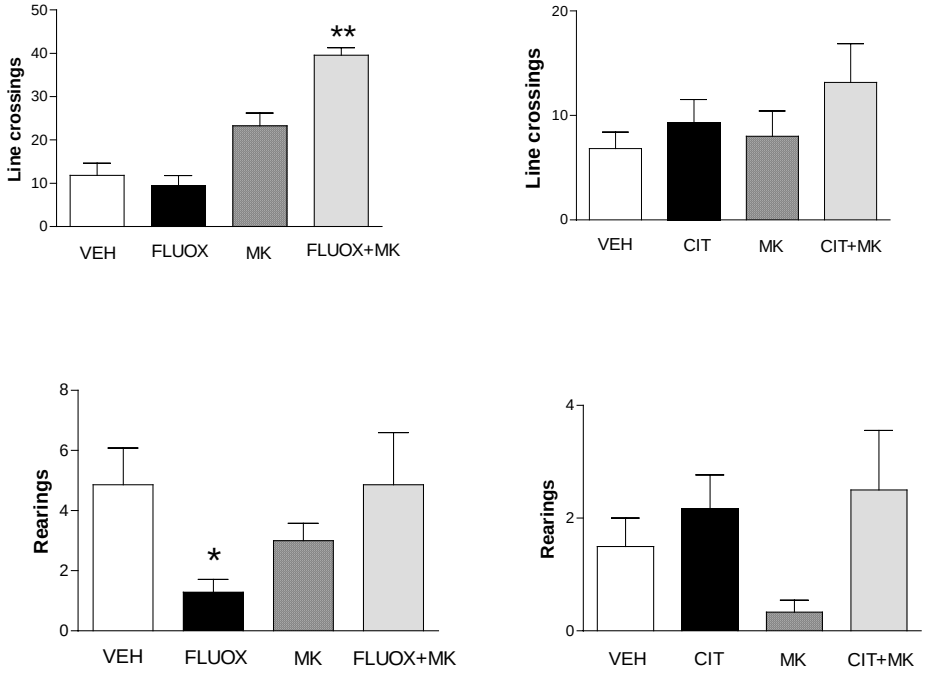

Figure 10. Effects of NMDA antagonist MK-801 on the effect of fluoxetine and citalopram in open field test. VEH = vechicle, FLUOX = fluoxetine 20 mg/kg, CIT = citalopram 5 mg/kg, MK = MK-801 0.1 mg/kg, FLUOX + MK = fluoxetine 20 mg/kg + MK-801 0.1 mg/kg, CIT + MK = citalopram 5 mg/kg + MK-801 0.1 mg/kg. All data are expressed as means±S.E.M. $^{*}p<0.05$, $^{**}p<0.01$ (Fisher's LSD test).

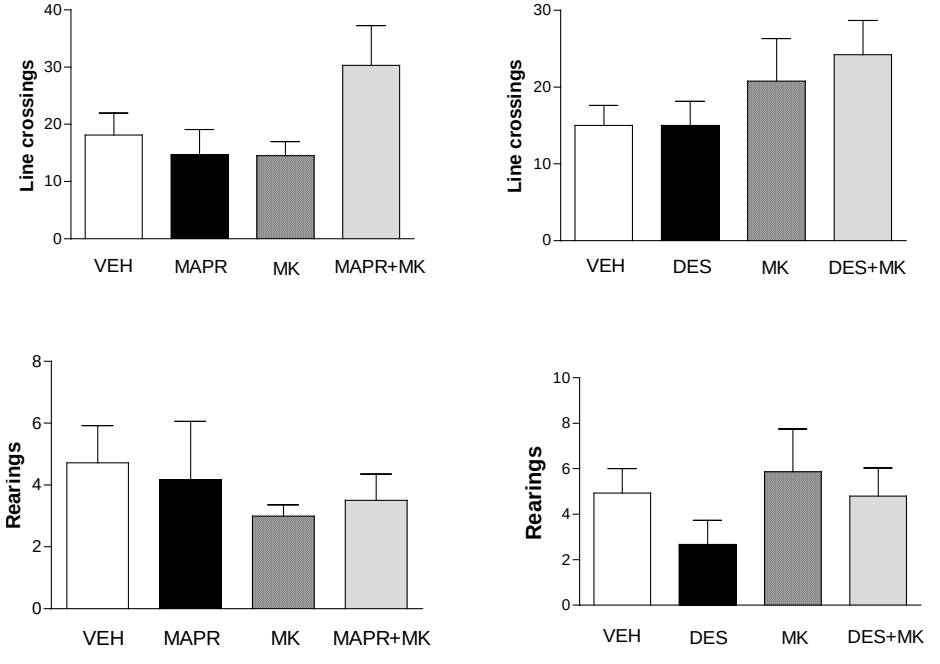

Figure 11. Effect of NMDA antagonist MK-801, maprotiline and desipramine in open field test. VEH = vechicle, MAPR = maprotiline 20 mg/kg, DES = desipramine 20 mg/kg, MK = MK-801 0.1 mg/kg, MAPR + MK = maprotiline 20 mg/kg + MK-801 0.1 mg/kg, DES + MK = desipramine 20 mg/kg + MK-801 0.1 mg/kg. All data are expressed as means±S.E.M. (Fisher's LSD test).

5.2.2. Forced swimming test

In the forced swimming experiments, where MK-801 treatment was combined with fluoxetine and citalopram, there were significant effects on immobility [$F(3,28)=12.8$, $p<0.001$, and $F(3,18)=3.7$, $p<0.05$, respectively]. No single drug treatment changed the immobility time in FST, but combination of MK-801 with fluoxetine or citalopram (Figure 12) reduced it. Combined treatment with MK-801 and maprotiline or desipramine was ineffective in this test (Figure 13). Thus in contrast to NARIs (maprotiline, desipramine), the combined treatment of MK-801 with SSRIs (citalopram, fluoxetine) elicit an antidepressant-like effect in FST.

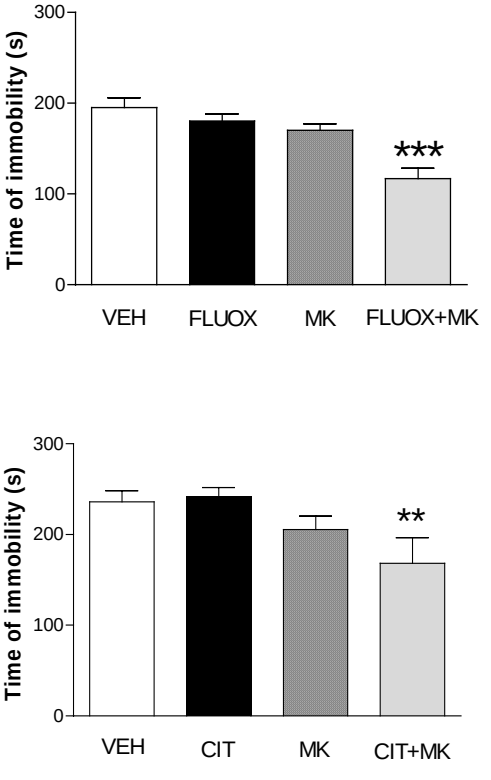

Figure 12. Effects of NMDA antagonist MK-801 on the effect of fluoxetine and citalopram on the forced swimming behaviour in rats**.** VEH = vechicle, FLUOX = fluoxetine 20 mg/kg, CIT = citalopram 5 mg/kg, MK = MK-801 0.1 mg/kg, FLUOX + MK = fluoxetine 20 mg/kg + MK-801 0.1 mg/kg, CIT + MK = citalopram 5 mg/kg + MK-801 0.1 mg/kg. All data are expressed as means±S.E.M**.** $^{**}p<0.01$, $^{***}p<0.001$ (Fisher's LSD test).

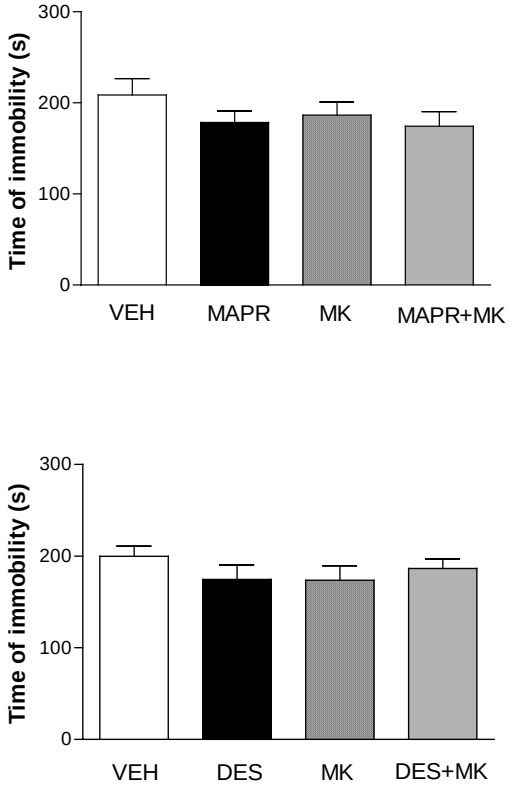

Figure 13. Effects of NMDA antagonist MK-801 on the effect of maprotiline and desipramine on the forced swimming behaviour in rats. VEH = vechicle, MAPR = maprotiline 20 mg/kg, DES = desipramine 20 mg/kg, MK = MK-801 0.1 mg/kg, MAPR + MK = maprotiline 20 mg/kg + MK-801 0.1 mg/kg, DES + MK = desipramine 20 mg/kg + MK-801 0.1 mg/kg. All data are expressed as means±S.E.M. (Fisher's LSD test).

5.3. Effects of antidepressants and 5-HT receptor ligands on brain monoamine content (Papers III–V)

The acute desipramine (10 mg/kg), trazodone (10 mg/kg) and citalopram (5 mg/kg) treatment had no major effect on NA (with one exception) or 5-HT (as well as 5-HIAA) content as compared with the vehicle-treated group. In the frontal cortex and hypothalamus dopamine content moderate changes were found. Citalopram reduced DOPAC and increased dopamine levels in frontal cortex. Trazodone reduced dopamine levels both in frontal cortex and hypothalamus (Table 10).

Statistically significant differences were found between the vehicle-treated and 1-NP (2 mg/kg) plus citalopram (5 mg/kg) treated groups in the 5-HIAA content in striatum: the 5-HIAA content was reduced after the combination treatment (Figure 14).

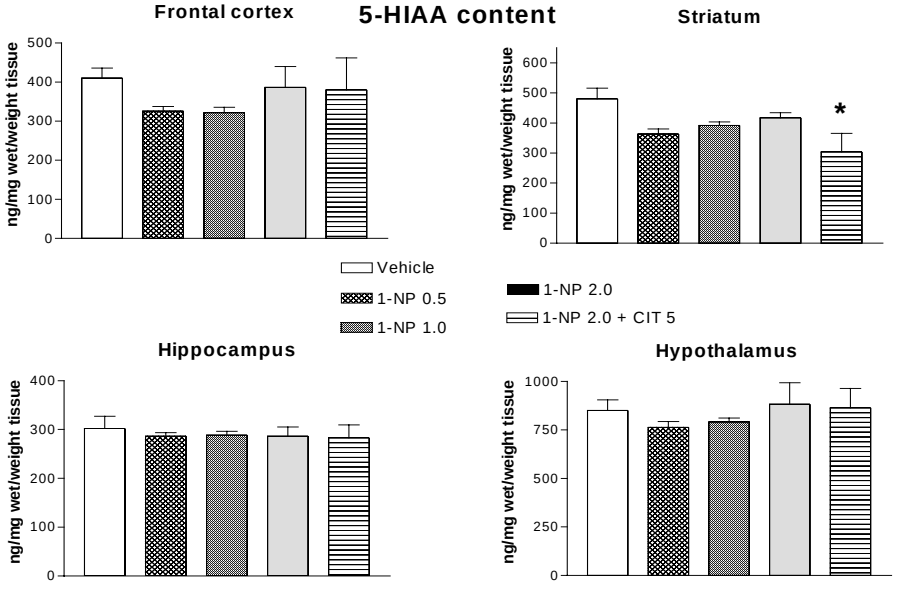

Figure 14. Effect of acute vehicle, 1-NP, or combined 1-NP+citalopram treatment on *post-mortem* 5-HIAA content in Wistar rats. The figures in the legend indicate the respective drug doses in mg/kg, i.p. $^{*}p<0.05$ as compared with the corresponding vehicle group (Fisher's LSD test after significant ANOVA).

In the frontal cortex, striatum, hippocampus, and hypothalamus, ANOVA revealed significant differences in the 5-HT and 5-HIAA levels in the p-CPA pretreated animals. Combined treatment with citalopram (5 mg/kg) and 8-OH-DPAT (0.1 mg/kg) increased the 5-HT and 5-HIAA content (Table 11).

Table 10. Effect of acute antidepressant treatment on monoamine content in four brain regions of rats subjected to forced swimming test.

Brain structure and treatment	Noradrenaline	DOPAC	Dopamine	5-HIAA	HVA	Serotonin
Frontal cortex						
Desipramine 10 mg/kg	95.7 ± 6.1	82.7 ± 10.5	110.9 ± 9.3	91.7 ± 2.7	76.6 ± 6.3	95.6 ± 5.7
Trazodone 10 mg/kg	105.5 ± 11.1	91.7 ± 7.8	67.7 ± 16.6*	95.9 ± 3.4	155.8 ± 23.0*	86.9 ± 4.9
Citalopram 5 mg/kg	100.0 ± 5.1	43.1 ± 5.4*	140.4 ± 14.6*	94.4 ± 4.4	87.2 ± 15.1	101.3 ± 9.1
Striatum						
Desipramine 10 mg/kg	131.1 ± 13.0	83.2 ± 6.4	93.8 ± 3.2	92.0 ± 3.6	75.7 ± 6.2	103.0 ± 3.0
Trazodone 10 mg/kg	121.1 ± 14.2	102.7 ± 5.1	79.9 ± 2.0	76.7 ± 4.3	101.3 ± 3.5	85.6 ± 1.4
Citalopram 5 mg/kg	52.2 ± 13.0*	82.8 ± 2.6	84.5 ± 2.0	71.8 ± 14.5	96.0 ± 1.7	82.0 ± 16.3
Hippocampus						
Desipramine 10 mg/kg	115.5 ± 7.7	89.9 ± 12.4	123.8 ± 29.8	85.5 ± 2.2	78.6 ± 12.1	76.8 ± 4.5
Trazodone 10 mg/kg	97.2 ± 9.1	124.8 ± 14.7	76.0 ± 9.8	109.4 ± 4.7	101.0 ± 3.2	84.0 ± 7.6
Citalopram 5 mg/kg	104.6 ± 6.4	110.4 ± 10.0	115.1 ± 13.6	82.5 ± 4.7	125.0 ± 10.0	101.6 ± 8.2
Hypothalamus						
Desipramine 10 mg/kg	86.5 ± 4.4	85.6 ± 5.3	90.1 ± 0.9	82.7 ± 7.3	69.0 ± 6.2*	90.4 ± 1.3
Trazodone 10 mg/kg	91.5 ± 11.2	125.7 ± 0.8	55.4 ± 2.2*	122.9 ± 4.7	102.3 ± 12.2	84.2 ± 3.8
Citalopram 5 mg/kg	91.61 ± 4.3	71.2 ± 5.5	71.4 ± 1.6	85.7 ± 6.8	77.8 ± 15.9	110.7 ± 7.2

All data (means±SEM, N per group = 5) are expressed as percentage from the respective vehicle-treated group, which was always considered 100%. *$p<0.05$ as compared with the corresponding vehicle group (100 %), Student's t-test.

Table 11. Effect of acute citalopram and 8-OH-DPAT treatment on the *post-mortem* 5-HT and 5-HIAA content in four brain regions

Brain structure	Pretreatmet	Drug treatment	5-HT	5-HIAA
FC	vehicle	vehicle	199.4 ± 11.1	297.2 ± 13.8
	vehicle	citalopram 5 mg/kg	208.5 ± 10.1	289.0 ± 8.2
	vehicle	8-OH-DPAT 0.1 mg/kg	208.6 ± 6.6	350.8 ± 14.9
	vehicle	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	205.3 ± 4.1	318.2 ± 7.9
	p-CPA	vehicle	28.4 ± 8.3	20.9 ± 7.0
	p-CPA	citalopram 5 mg/kg	32.0 ± 9.4	31.9 ± 8.4
	p-CPA	8-OH-DPAT 0.1 mg/kg	20.8 ± 4.3	15.1 ± 1.8
	p-CPA	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	129.3 ± 59.1*	129.8 ± 59.8*
STR	vehicle	vehicle	213.1 ± 6.1	430.8 ± 19.3
	vehicle	citalopram 5 mg/kg	224.9 ± 5.3	396.8 ± 4.6
	vehicle	8-OH-DPAT 0.1 mg/kg	207.5 ± 17.5	425.7 ± 45.1
	vehicle	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	233.5 ± 9.4	407.1 ± 14.3
	p-CPA	vehicle	34.1 ± 8.0	40.8 ± 6.3
	p-CPA	citalopram 5 mg/kg	40.9 ± 13.6	43.4 ± 9.3
	p-CPA	8-OH-DPAT 0.1 mg/kg	24.7 ± 2.1	29.6 ± 1.8
	p-CPA	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	133.3 ± 62.7*	204.9 ± 99.7*

Brain structure	Pretreatmet	Drug treatment	5-HT	5-HIAA
HI	vehicle	vehicle	203.1 ± 50.0	290.3 ± 14.8
	vehicle	citalopram 5 mg/kg	110.1 ± 19.8	218.7 ± 36.7
	vehicle	8-OH-DPAT 0.1 mg/kg	131.5 ± 3.5	348.6 ± 23.1
	vehicle	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	125.3 ± 8.7	296.5 ± 14.6
	p-CPA	vehicle	14.9 ± 4.1	15.4 ± 4.4
	p-CPA	citalopram 5 mg/kg	13.4 ± 4.6	18.9 ± 5.8
	p-CPA	8-OH-DPAT 0.1 mg/kg	8.2 ± 0.6	7.5 ± 0.8
	p-CPA	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	99.8 ± 58.3*	108.6 ± 56.1*
HY	vehicle	vehicle	321.1 ± 8.8	715.8 ± 27.7
	vehicle	citalopram 5 mg/kg	327.8 ± 18.5	650.8 ± 41.8
	vehicle	8-OH-DPAT 0.1 mg/kg	384.5 ± 11.0	636.3 ± 49.8
	vehicle	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	287.8 ± 25.7	578.8 ± 39.8
	p-CPA	vehicle	52.3 ± 13.3	50.7 ± 16.1
	p-CPA	citalopram 5 mg/kg	59.6 ± 13.7	66.1 ± 11.4
	p-CPA	8-OH-DPAT 0.1 mg/kg	46.3 ± 4.9	37.6 ± 4.7
	p-CPA	citalopram 5 mg/kg + 8-OH-DPAT 0.1 mg/kg	197.5 ± 94.6*	292.7± 107.7*

All data (N per drug treatment group = 5) are expressed as ng/g wet weight tissue (means±SEM). *P<0.05 as compared with the corresponding p-CPA + vehicle group (Fisher's LSD test). FC – frontal cortex, STR – striatum, HI – hippocampus, HY – hypothalamus.

5.4. The effect of 5-HT$_{1A}$ agonist 1-NP in the *in vivo* microdialysis study (Paper IV)

Using the *in vivo* microdialysis technique, no difference in the 5-HT or 5-HIAA output between the treatment groups: vehicle, 1-NP (2 mg/kg) and 1-NP (2 mg/kg) with citalopram (5 mg/kg), was found in the frontal cortex (Figure 15).

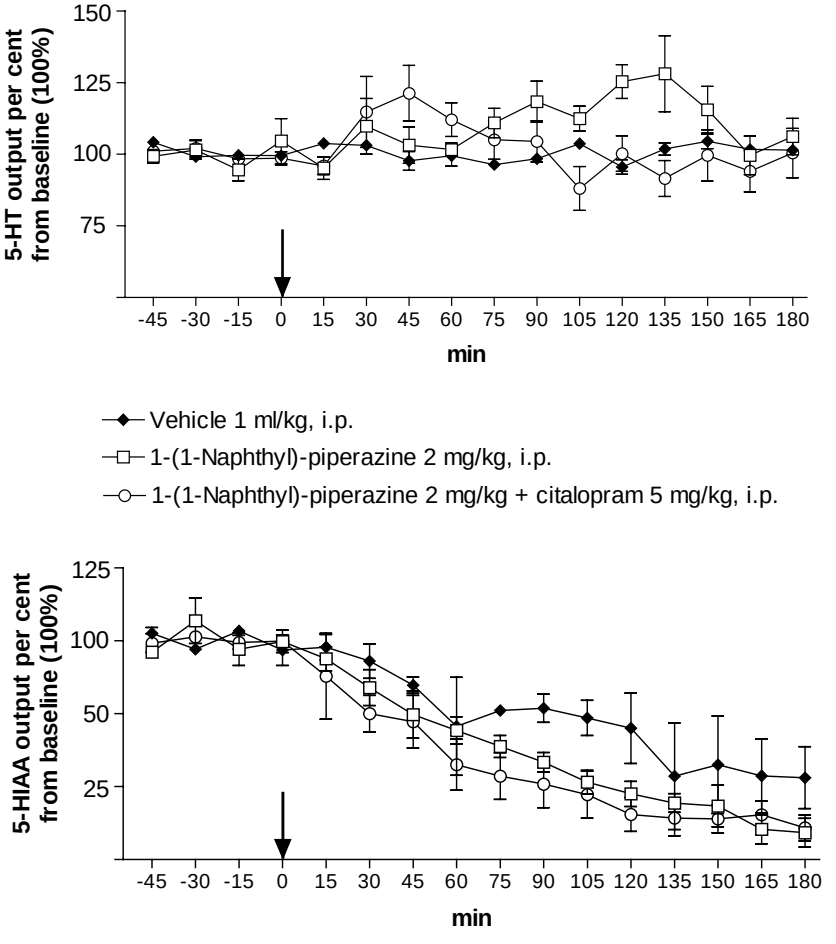

Figure 15. Effect of acute vehicle, 1-NP, or combined 1-NP+citalopram treatment on frontal cortex 5-HT and 5-HIAA content in chloral hydrate anaesthetised Wistar rats: An *in-vivo* microdialysis experiment. The arrow indicates the time point of i.p. drug injection. No significant differences in repeated measures ANOVA in the treatment factor.

6. DISCUSSION

One of the most selective SSRIs is citalopram, which exists as a racemic mixture of R- and S-enantiomers. The S-enantiomer escitalopram is the therapeutically active portion of the parent compound and has a proven quick and well-pronounced antidepressant efficacy. The R-enantiomer lacks activity as an antidepressant and has been shown to inhibit the effect of the S-enantiomer when the two are combined (Malin et al., 2004; Kennedy et al., 2006). Although citalopram is not considerably superior in its antidepressive properties as compared with other SSRIs, citalopram is widely used as a reference drug of SSRIs (Åsberg and Mårtenson, 1993; De Jonghe and Swinkels, 1997). Desipramine is used often as a reference drug of NARIs.

Using the open field test and plus-maze test, we found that acute antidepressant treatment elicits an antiexploratory effect. Desipramine and maprotiline elicit more pronounced antiexploratory effect, citalopram treatment has of weak effect. The latter is in agreement with some previous reports showing that small doses of SSRIs do not affect exploratory behaviour (Chopin et al., 1987; Handley et al., 1993). In good agreement with our previous experiments, desipramine treatment reduced the time of immobility in FST, while citalopram treatment was ineffective (Skrebuhhova et al., 1999; Rudissaar et al., 2001).

The acute antidepressant treatment had no major effect on NA or 5-HT (as well as 5-HIAA) content as compared with the vehicle-treated group. It has been hypothesised that in fact, acute antidepressant treatment does not increase the monoamine contents in synaptic clefts but elevates it mainly in the extracellular fluid.

6.1. Role of 5-HT_{1A} receptors in the action of antidepressants

Our results indicate that acute citalopram (5 mg/kg) treatment was ineffective both in the open field and FST. There was no significant effect of citalopram in FST even in doses 10–20 mg/kg. Since the effect of serotoninergic drugs may depend on the function of the baseline activity of the neurotransmission, we applied an additional factor, para-chlorophenylalanine (p-CPA) pretreatment. Acute p-CPA (350 mg/kg) treatment depletes almost 90 per cent of the 5-HT stores for at least 72 hours (Skrebuhhova et al. 1999), thereby giving an additional opportunity for the development of (mainly postsynaptic) sensitization of the 5-HT receptors. We found, though there were some differences in the absolute values of the exploratory behaviours criteria measured, that trends were similar in the vehicle and the p-CPA pretreated groups. In the FST, none of the drug treated groups was different from the vehicle group, although between the drug treatment groups differences were found. It can be concluded

that in the tests of exploratory behaviour the additional activation of 5-HT_{1A} receptors by 8-OH-DPAT has a greater value than in a condition of heavy stress such as the FST.

In the open field test, in the present study a clear anxiolytic-like effect of acute 1-NP treatment was found as indicated by the increased number of line crossings and increased sum of exploratory events. Citalopram did not change the anxiolytic-like effect of 1-NP treatment, though a trend toward a further increase was found. The simple explanation is that the number of exploratory events had reached already the maximum and could not be increased any more. In the social exploration test, no statistically significant differences could be detected, however, a tendency toward the prolongation of the social interaction as a consequence of the 1-NP treatment was found which finding is in agreement with the experiments of Kennett (Kennett, 1992).

Acute administration of the 5-HT_{1A} receptor antagonist WAY 100635 alone did not induce any effect on the immobility time and had anxiolytic-like activity in the open field test. However, WAY 100635 treatment potentiated the anxiogenic-like effect of desipramine and fluoxetine, but not with citalopram in the open field and FST. This effect of combined treatment on the forced swimming can be explained by the ability of WAY 100635 to increase the amount of 5-HT in the synaptic cleft by blocking the presynaptic 5-HT_{1A} receptors. This indicates that the effects of adrenergic antidepressants are not mediated through 5-HT_{1A} receptors. Concomitant treatment with citalopram did not elicit a similar effect, because it increases the level of 5-HT similarly to WAY 100635.

On the basis of the behavioural data it was expected that the *post-mortem* 5-HT or 5-HIAA content of four brain regions of animals should not be changed. This was true for the vehicle-pretreated animals, but interestingly the combined citalopram and 8-OH-DPAT treatment partially reversed the reduction of 5-HT and 5-HIAA content induced by p-CPA treatment. This could be interpreted as 5-HT receptor inhibition elicited by citalopram. It seems that quite large variation in data to might be due to the individual differences in response to the combined citalopram and 8-OH-DPAT treatment. Nevertheless, it should be borne in mind that this partial reversal of the decrease of 5-HT and 5-HIAA content is not reflected in the time of immobility in the FST. Consequently, it can be proposed that although the 5-HT_{1A} receptors are involved in the mediation of behavioural phenomena in rats, in the FST the 5-HT receptors other than the 5-HT_{1A} receptor subtype are the critical substrates in the mediation of behaviour. This conclusion is also in line with the report by Redrobe and Bourin (1998), who found that the 5-HT_2 receptors rather than 5-HT_{1A} receptors play a key role in the mediation of the behavioural elements of forced swimming behaviour.

The only statistically significant decrease in the 5-HIAA content in striatum (1-NP+citalopram group *versus* vehicle group) can be interpreted as a sign of decreased release and metabolism of 5-HT in this brain structure, elicited by

agonistic effect of 1-NP on 5-HT_1 receptors. However, whether this statistically significant decrease has a biologically meaningful value remains to be elucidated. The *in vivo* microdialysis experiment demonstrated that there is no difference between these treatment groups with regard to the 5-HT and 5-HIAA output.

An explanation could be, that the acute administration of 1-NP induces only changes on the receptor level and the changes in the 5-HT content can be seen only after a repeated administration. Alternatively, it cannot be excluded, that there were moderate but opposite changes in the 5-HT metabolism within the different parts of a brain structure. In such a case, we were unable to detect any changes, since in the *post-mortem* experiments we pooled all tissue of the respective brain area into one sample.

Another important problem, which remains still unanswered is the role of the 5-HT_{1A} somatodendritic autoreceptors. In general, it is believed that antidepressant treatment desensitizes the 5-HT_{1A} autoreceptors after prolonged administration (Hervas et al., 2001). The drugs acting at the 5-HT_{1A} receptors are not exclusively selective for the 5-HT receptor subtypes, while, indeed, they are neither selective for the autoreceptors. In some cases, the dual (or multiple) action of a 5-HT-ergic compound (*e.g.*, 1-NP) is beneficial in terms of induction of behavioural effects, but on the other hand does not allow to distinguish between the roles of different receptor subtypes.

6.2. Role of 5-HT_{2A} receptors in the action of antidepressants

The forced swimming study indicates that the 5-HT_{2A} receptors agonist DOI elicited an antidepressant-like effect. Citalopram treatment was ineffective which finding is in line with earlier reports (Borsini, 1995). It is noteworthy that citalopram treatment was partially able to reverse the effect of DOI. Because citalopram does not have any affinity to the 5-HT_{2A} receptors, this partial effect is evidently due to the increased serotonin extracellular levels which activates the 5-HT_{1A} receptors and inhibits the 5-HT neurons activity. The activation of the 5-HT_{1A} receptors, indeed, may lead to the inhibition of 5-HT release and to the prolongation of the immobility (Borsini, 1995). Furthermore, the effect of other 5-HT receptor subtypes could also be implicated in this effect as serotonin is the endogenous ligand for all 5-HT receptor subtypes. However, this finding further confirms that the 5-HT_{2A} receptors might be partially involved in the mechanism of action of antidepressants.

Our results from the elevated plus-maze test indicate that desipramine in a dose of 10 mg/kg elicits an antiexploratory effect. Citalopram 5 mg/kg treatment was ineffective, and this result is in line with some reports where the SSRIs have been ineffective in the elevated plus-maze, but not in the other tests of exploratory behaviour (Chopin and Briley, 1987; Handley and McBlane,

1993). The acute 5-HT_{2A} receptor antagonist (ketanserin, ritanserin) challenge manifested the antiexploratory effect of citalopram while the most prominent effect was found by ritanserin challenge. Because both 5-HT_{2A} receptor antagonists in itself were without any effect, this finding provides evidence that the reported antiexploratory effect of the SSRIs may be attenuated, at least partially, via the 5-HT_{2A} receptors. However, the combined antidepressant plus 5-HT_{2A} receptor antagonist treatment did not change either the open/total arm entries or open/total time ratio, which finding indirectly indicates that these interactions finding may be rather related to motivation than anxiety.

In the FST, desipramine and maprotiline treatment reduced the time of immobility. On the second day of the experiment 5-HT_{2A} receptor antagonists were ineffective and did not further reduce the time of immobility in combination with desipramine or citalopram but a tendency toward this direction was found. The latter finding is in line with the results from Redrobe and Bourin (1997), who proposed that the 5-HT_{2A} receptors have some role in the mediation of the anti-immobility effect of antidepressants. Furthermore, this finding indicates also that the suppression of the exploratory behaviour in the elevated plus-maze test is not due to the sedative effect of these drug combinations.

The FST consists of several components such as fear of soaking, physical exercise, hopelessness, *etc*., that sum up in heavy stress. It can be predicted that under these conditions the SSRIs and 5-HT_{2A} receptor antagonist interactions could be more pronounced. As an additional factor, the DSP-4 (a selective noradrenergic neurotoxin) pretreatment was applied. DSP-4 treatment has been proposed to be a chemical model of affective disorders (Ross and Renyi, 1976; Fritschy and Grzanna, 1989; Harro et al., 1995) and therefore, it provides additional information on the mechanism of action of the antidepressants. In the DSP-4 pretreated animals, neither desipramine nor citalopram in combination with 5-HT_{2A} receptor antagonists reduced the time of immobility. This finding indicates that noradrenergic denervation may reverse the antiimmobility effect of noradrenergic antidepressants. The DSP-4 pretreatment experiments demonstrate that the noradrenergic neurotransmission plays a key role in the FST and the time of immobility *resp*. mobility is dependent on the state of the noradrenergic pathways. In addition, the elevated plus-maze and FST results indicate that the involvement of the 5-HT_{2A} receptors in mediating the behavioural phenomena of the antidepressant treatment is dependent on the capability of procedures to induce aversive stimuli. Thus, in the elevated plus-maze, but not FST some of the behavioural effects of the SSRI treatment may be mediated via the 5-HT_{2A} receptors.

6.3. Role of 5-HT_3 receptors in the action of antidepressants

The 5-HT_3 receptors seem not to be involved in the neurobiology of forced swimming behaviour, because in the FST neither ondansetron alone nor in combination with citalopram had any effect on the time of immobility. Further, the open field test confirms that this dose of ondansetron was sufficient to evoke CNS-mediated behavioural phenomena (the dose of 10 mg/kg of the mCPBG treatment was found to be effective in our previous study (Rudissaar et al., 1999), therefore it was not studied in these series of experiments). In the plus-maze test ondansetron increased the time and percentage of time spent in open arms more than two-fold, indicating its clear anxiolytic-like properties. Other criteria, i.e. open and total arm entries and line crossing, did not differ from the control group. This can be explained on the basis that distinct criteria of the plus-maze behaviour reflect distinct components of exploratory behaviour. Thus, the time spent exploring is related to the anxiety of an animal, and the other criteria to the novelty-related drive. Furthermore, because ondansetron did not increase open field behaviour, it can speculated that the 5-HT_3 receptors mediate the anxiety-related, but not novelty-related, component of exploratory behaviour. A persuasive explanation is, indeed, that this combination of drugs may impair the motivational component of exploration. Thus the 5-HT_3 receptor-controlled DA overflow in the CNS (Benloucif et al., 1993) is suppressed by ondansetron, which is not sufficient alone to reduce the motivational component of exploratory behaviour. As a consequence, if the antidepressants-induced increase in NA and/or 5-HT content in the synaptic cleft, followed by the activation of distinct recetor subtypes, might lead to the motivational suppression because of the relative DA deficit is a secondary effect. Furthermore, anxiolytic-like effect of ondansetron does not compensate the diminished motivation either.

6.4. Role of glutamatergic system in the action of antidepressants

MK-801 (dizocilpine) is known to induce a dose-dependent locomotor hyper-activity in rodents (Liljequist et al., 1991; Carey et al., 1998; Andine et al., 1999). Our results indicated that acute administration MK-801 at doses 0.025, 0.05 and 0.1 mg/kg did not affect locomotor activity significantly in the open field test. This is consistent with previous findings that describe an increase in the locomotor behaviour in rats following administration of MK-801 beginning from the dose 0.2 mg/kg (Carey et al., 1998; Andine et al., 1999).

FST is a behavioral test in rodents that predicts the clinical efficacy of many types of antidepressant treatments (Porsolt et al., 1979). Previous studies have demonstrated that acute administration of MK-801 at the dose 0.1, but not

0.05 mg/kg elicits an antidepressant-like action in the classic version of the FST (Maj et al., 1992). In the present study, drugs were administered acutely before the single forced swimming session, as is customary for the mouse version of the FST (Borsini, 1995; Petit-Demouliere et al., 2005). This was done in order to find out whether the NMDA receptor blockade would help to reveal antidepressant action after a single treatment. In such conditions, MK-801 (0.1 mg/kg) did not reduce the immobility time in the FST in rats. As expected, none of the antidepressants used had any independent effect on the forced swimming. However, combined treatment with MK-801 and SSRIs citalopram and fluoxetine shortened immobility and thus had an antidepressant-like effect.

It is well known that SSRIs do not show antidepressant-like activity in the classic version of the FST (Borsini et al., 1988; Borsini, 1995). Furthermore, in rats the anti-immobility effect of antidepressants is usually observed after administration of the drug more than once. Antagonism at the NMDA receptors, however, revealed the antidepressant-like effect of fluoxetine and citalopram. In contrast, combined treatment with MK-801 and NARIs, desipramine or maprotiline, had no effect in the FST. This suggests that NMDA receptor activation elicited by swimming stress does not mask the antidepressant-like effect in FST of primarily noradrenergic drugs.

It has been found previously that antidepressants can potentiate the increase in locomotor activity induced by MK-801. A single dose of fluoxetine (10 mg/kg) increased the locomotor hyperactivity induced by MK-801 (Maj et al., 1996). Furthermore, both a NARI (desipramine) and a SSRI (citalopram) have been shown to enhance the MK-801-induced locomotor effect (Maj et al., 1991). In the present study, only the combination of fluoxetine and MK-801 enhanced locomotor activity significantly over baseline. As the more serotonin selective antidepressant citalopram together with MK-801 did not enhance locomotor activity, the potentiating effect of MK-801 on antidepressant-like action of SSRIs in the FST appears not to be due to a nonspecific psychostimulant effect.

Similar effects of SSRIs have also been described for other NMDA receptor antagonists (PCP) in the classic FST (Rogoz et al., 2002). The present results suggest that by blocking the NMDA receptors an antidepressant effect can be revealed with the FST using only one swimming session and single drug administration, as is the routine in antidepressant drug screening in mice, but this holds only for drugs acting more selectively on serotonergic neurons. This suggests that differences in mice and rats regarding the optimal forced swimming procedure may be based on differences in recruitment of serotonergic activity by swimming stress.

The mechanisms through which NMDA receptor antagonists potentiated the antidepressive effect of SSRIs are not yet clear. It is well-established that acute administration of MK-801 activates serotonergic neurons and increases extracellular 5-HT in several brain regions of rats (Locher et al., 1991; Whitton

et al., 1992; Yan et al., 1997; Callado et al., 2000). Thus serotonergic neurotransmission seems to be significantly involved in the behavioural effects induced by MK-801 (Locher and Honack, 1992). Acute admistration of SSRIs is increasing the extracellular levels of 5-HT (Kreiss and Lucki, 1995; Felton et al., 2003), and administration of MK-801 together with SSRIs would produce synergistic effect in raising the extracellular concentration of 5-HT. This suggests that interaction between glutamatergic and serotonergic systems in the brain may be significant in the aetiology of depression.

7. CONCLUSIONS

The experimental results lead to the following conclusions:

1.1 SSRIs, by increasing the extracellular level of 5-HT, elicit or increase anxiety by the effect of serotonin on various subtypes of postsynaptic 5-HT receptors and by decrease of firing of 5-HT neurons through negative feedback. The anxiogenic-like effect of noradrenergic antidepressants (desipramine, maprotiline) does not involve the 5-HT_1 or 5-HT_2 receptors.

1.2. Drugs inhibiting the activity of serotonergic neurotransmission, such as 8-OH-DPAT (an agonist of somatodendritic 5-HT_{1A} autoreceptors) and 1-NP (an agonist on somatodendritic 5-HT_{1A} receptors and antagonist of the 5-HT_2 receptors) have anxiolytic effect by preventing additional release of serotonin or blocking postsynaptic 5-HT_2 receptors. Noradrenergic antidepressants, but not SSRIs, are antagonizing the effects of 1-NP.

2. The increased availability of serotonin on 5-$HT_{2A/2C}$ receptors increases anxiety in exploratory behaviour based tests and could be responsible in shortening of immobility time in forced swim test. The agonist of 5-HT_2 receptors DOI is shortening the immobility time as antidepressants. The noradrenergic antidepressants (desipramine, maprotiline) do not shorten the immobility time in FST by a serotonergic mechanism. The shortening of immobility time in FST seems to depend mostly on adrenopositive effect of drugs.

5-HT_3 receptors may modulate the open field behaviour but do not have a major role in the forced swimming test.

3. Acute antidepressant treatment has no major effect on 5-HT and 5-HIAA content as compared with the vehicle-pretreated animals. The combined citalopram and 8-OH-DPAT treatment partially reversed the p-CPA-induced decrease of 5-HT and 5-HIAA content but this effect is not reflected in the time of immobility in the FST. The present results indicate that although the 5-HT_{1A} receptors are involved in the mediation of behavioural phenomena in rats, in the FST the 5-HT receptors other than the 5-HT_{1A} receptor subtype are the critical substrates in the mediation of behaviour.

4. NMDA receptor blockade reveals behaviorally activating effects of acute admistration of serotonergic antidepressants. The present results indicate that co-administration of SSRIs with the NMDA receptor antagonists may induce a faster and more pronounced antidepressive activity than treatment with antidepressants alone. The NMDA antagonists do not potentiate the antidepressant-like effects of NARIs.

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9. SUMMARY IN ESTONIAN

Antidepressantide toimemehhanism: serotoniinergiline süsteem ja tema interaktsioon glutamaadiga

Antidepressandid, nii monoamiini oksüdaasi inhibiitorid (MAOI-d), tritsüklilised antidepressandid (TTA-d), valikulised noradrenaliini tagasihaarde inhibiitorid (VNTI-d) kui ka valikulised serotoniini tagasihaarde inhibiitorid (VSTI-d), on tänapäeval psühhotroopsetest ainetest kõige enam kasutatavad. Nad on leidnud kasutamist mitte ainult depressiivsete häirete vaid ka paanilise hirmu, ärevushäirete, hüperagressiivsuse jt. seisundite ravis. Depressiooni väljakujunemisel omavad suurt tähtsust häired neuronaalsetes monoamiinergilistes süsteemides, millest olulisemaks peetakse häiret serotoniinergilises (5-HT) süsteemis. Ained, mis toimivad 5-HT_{1A} ja 5-$HT_{2A/2C}$ retseptorite kaudu, võivad mõjutada depressiooni kulgu. Samuti tekivad depressiooni korral muutused N-metüül-D-aspartaat (NMDA) retseptorkompleksis frontaalkoores ja hipokampuses. Antidepressantide manustamine langetab glütsiini sidumiskohtade afiinsust NMDA retseptorkompleksis, mida võib tõlgendada kui retseptori funktsiooni langust. Samuti on näidatud NMDA retseptori antagonistide võimalikku antidepressiivset toimet.

Antud töös võrdlesime valikuliste serotoniini tagasihaarde inhibiitorite (VSTI-d, tsitalopraam, fluoksetiin) ja valikuliste noradrenaliini tagasihaarde inhibiitorite (VNTI-d, desipramiin, maprotiliin) toimet serotoniinergilisse süsteemi. Uurisime nende toime muutusi 5-HT_{1A} retseptorite agonistide (8-OH-DPAT, 1-NP) ja antagonisti (WAY 100635), 5-HT_{2A} retseptorite agonisti (DOI) ja antagonistide (ketanseriin, ritanseriin) ning 5-HT_3 retseptorite agonisti (m-CPBG) ja antagonisti (ondansetroon) foonil. Samuti oli eesmärgiks selgitada erinevate antidepressantide toimet NMDA retseptori antagonisti MK-801 (dizocilpine) toimele käitumiskatsetes. Katsed teostasime Wistar-liini rottidel. Uurimismeetoditest kasutasime avarvälja testi, pluss-puuri testi, sundujumise (Porsolt`) testi, *post-mortem* monoamiinide määramist ja mikrodialüüsi metoodikat.

Antidepressantide akuutne manustamine annustes 5–15 mg/kg i.p. omas annusest sõltuvat uudistavat käitumist pärssivat toimet nii avarvälja kui ka pluss-puuri testis, kusjuures VNTI-de toime oli enam väljendunud. 5-HT_{1A} agonist 8-OH-DPAT (0.1 mg/kg) omas uudistavat käitumist pärssivat toimet, 5-HT_{1A} antagonist WAY 100635 (0.3 mg/kg) omas nõrka anksiolüütilist toimet. 5-$HT_{2A/2C}$ retseptorite antagonistid ketanserin ja ritanserin (3 mg/kg) ei omanud olulist toimet pluss-puuri testis kuid kombinatsioonis tsitalopraamiga (5 mg/kg) intensiivistasid viimase uudistavat käitumist pärssivat toimet. 5-HT_3 retseptori antagonisti ondansetrooni (4 mg/kg) akuutne manustamine pluss-puuri testis tõi esile anksiolüütilise efekti, mis väljendus suurenenud väljumistega avatud säärele. Tsitalopraam (5 mg/kg) ja desipramiin (10 mg/kg)

kõrvaldasid ondansetrooni (4 mg/kg) efekti, vähendades väljumiste arvu avatud säärele. Sundujumise katsete tulemused näitasid, et VNTI-d (desipramiin ja maprotiliin) ja 5-HT_2 agonist DOI lühendasid passiivsuse perioodi kestvust sundujumise katses. VSTI tsitalopraam, samuti 5-HT_2 antagonistid ketanseriin ja ritanseriin ning 5-HT_3 antagonist ondansetroon ei mõjutanud immobiilsust. 5-HT_2 ja 5-HT_3 retseptorite agonistide passiivsuseperioodi lühendav toime esines ka antidepressantide kasutamise foonil, kusjuures antagonistid antidepressantide toimet ei mõjutanud. Seega võib väita et muutused serotoniini vabanemises on seotud VSTI-de toimega käitumisaktiivsusele. Suurenenud serotoniini vabanemine põhjustab 5-HT_{2A} retseptori vahendusel suurenenud ärevust, pärssides käitumisaktiivsust ja lühendades sundujumise testis passiivsuseperioodi.

NMDA antagonist MK-801 annustes 0,025–0,1 mg/kg i.p. ei mõjutanud liikumisaktiivsust avarvälja testis ega immobiilsuse aega sundujumise testis. MK-801 kombinatsioonis VSTI fluoksetiiniga, omas käitumisaktiivsust suurendavat toimet, samuti lühenes koosmanustamisel VSTI-dega immobiilsuse aeg. Samas ei muutnud MK-801 desipramiini toimet käitumisaktiivsusele ja immobiilsuse ajale. Seega VSTI-d potentseerivad NMDA retseptori antagonisti MK-801 toimeid käitumisele.

Järeldused

1.1. VSTI-d, suurendades 5-HT ekstratsellulaarset taset, mis omakorda mõjutades postsünaptilisi 5-HT retseptoreid, põhjustavad või suurendavad ärevust katseloomadel. VNTI-de (desipramiin, maprotiliin) ärevust suurendav toime ei ole vahendatud 5-HT_1 ja 5-HT_2 retseptorite kaudu.

1.2. Ained, mis inhibeerivad serotoniinergilise närviülekande aktiivsust, nagu 8-OH-DPAT (5-HT_{1A} autoretseptorite agonist) ja 1-NP (5-HT_{1A} autoretseptorite agonist ja 5-HT_2 retseptorite antagonist), omavad anksiolüütilist toimet takistades 5-HT vabanemist või blokeerides postsünaptilised 5-HT_2 retseptorid.

2. Serotoniini suurenenud toime 5-$HT_{2A/2C}$ retseptoritele põhjustab ärevuse suurenemist käitumiskatsetes ning võib põhjustada immobiilsuse aja lühenemist sundujumise katses. 5-HT_2 retseptorite agonist DOI lühendab immobiilsuse aega sarnaselt antidepressantidele. VNTI-d (desipramiin, maprotiliin) ei põhjusta immobiilsuse vähenemist serotoniinergiliste mehhanismide kaudu. Immobiilsuse aja lühenemine on peamiselt põhjustatud adrenopositiivsetest toimetest.

5-HT_3 retseptorid võivad mõjutada käitumist avarvälja katses, kuid ei oma olulist rolli sundujumise testi (akuutse stressi) korral.

3. Akuutne antidepressantide manustamine ei mõjutanud *post-mortem* 5-HT ja 5-HIAA sisaldust võrreldes kontrollgrupiga. Tsitalopraami ja 8-OH-DPAT koosmanustamine kõrvaldas p-CPA-indutseeritud 5-HT ja 5-HIAA sisalduse vähenemise kuid see toime ei väljendunud immobiilsuse aja muutuses sundujumise katses. Käesolev tulemus näitab, et kuigi 5-HT_{1A} retseptorid mõjutavad käitumist ei oma nad olulist rolli sundujumise testis.

4. NMDA retseptorite blokaad avaldab VSTI-de foonil käitumist aktiveerivat toimet. See tulemus näitab, et VSTI-de koosmanustamine koos NMDA antagonistidega võib kiirendada antidepressiivse toime saabumist ja omada suuremat antidepressiivset aktiivsust. NMDA antagonistid ei potentseeri VNTI-de antidepressiivset toimet.

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